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(54) Title: MICRORNA MOLECULES

(57) Abstract: In *Caenorhabditis elegans*, lin-4 and let-7 encode 22- and 21-nucleotide RNAs, respectively, that function as key regulators of developmental timing. Because the appearance of these short RNAs is regulated during development, they are also referred to as "small temporal RNAs" (stRNAs). We show that many more 21- and 22-nt expressed RNAs, termed microRNAs, (miRNAs), exist in invertebrates and vertebrates, and that some of these novel RNAs, similar to let-7 stRNA, are also highly conserved. This suggests that sequence-specific post-transcriptional regulatory mechanisms mediated by small RNAs are more general than previously appreciated.

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MicroRNA molecules**Description**

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The present invention relates to novel small expressed (micro)RNA molecules associated with physiological regulatory mechanisms, particularly in developmental control.

10 In *Caenorhabditis elegans*, *lin-4* and *let-7* encode 22- and 21-nucleotide RNAs, respectively (1, 2), that function as key regulators of developmental timing (3-5). Because the appearance of these short RNAs is regulated during development, they are also referred to as "microRNAs" (miRNAs) or small temporal RNAs (stRNAs) (6). *lin-4* and *let-21* are the only known
15 miRNAs to date.

Two distinct pathways exist in animals and plants in which 21- to 23-nucleotide RNAs function as post-transcriptional regulators of gene expression. Small interfering RNAs (siRNAs) act as mediators of sequence-specific mRNA degradation in RNA interference (RNAi) (7-11) whereas
20 miRNAs regulate developmental timing by mediating sequence-specific repression of mRNA translation (3-5). siRNAs and miRNAs are excised from double-stranded RNA (dsRNA) precursors by Dicer (12, 13, 29), a multidomain RNase III protein, thus producing RNA species of similar size.
25 However, siRNAs are believed to be double-stranded (8, 11, 12), while miRNAs are single-stranded (6).

We show that many more short, particularly 21- and 22-nt expressed RNAs, termed microRNAs (miRNAs), exist in invertebrates and vertebrates,
30 and that some of these novel RNAs, similar to *let-7* RNA (6), are also highly conserved. This suggests that sequence-specific post-transcriptional

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regulatory mechanisms mediated by small RNAs are more general than previously appreciated.

The present invention relates to an isolated nucleic acid molecule comprising:

(a) a nucleotide sequence as shown in Table 1, Table 2, Table 3 or Table 4

(b) a nucleotide sequence which is the complement of (a),

(c) a nucleotide sequence which has an identity of at least 80%, preferably of at least 90% and more preferably of at least 99%, to a sequence of (a) or (b) and/or

(d) a nucleotide sequence which hybridizes under stringent conditions to a sequence of (a), (b) and/or (c).

In a preferred embodiment the invention relates to miRNA molecules and analogs thereof, to miRNA precursor molecules and to DNA molecules encoding miRNA or miRNA precursor molecules.

Preferably the identity of sequence (c) to a sequence of (a) or (b) is at least 90%, more preferably at least 95%. The determination of identity (percent) may be carried out as follows:

$$I = n : L$$

wherein I is the identity in percent, n is the number of identical nucleotides between a given sequence and a comparative sequence as shown in Table 1, Table 2, Table 3 or Table 4 and L is the length of the comparative sequence. It should be noted that the nucleotides A, C, G and U as depicted in Tables 1, 2, 3 and 4 may denote ribonucleotides,

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deoxyribonucleotides and/or other nucleotide analogs, e.g. synthetic non-naturally occurring nucleotide analogs. Further nucleobases may be substituted by corresponding nucleobases capable of forming analogous H-bonds to a complementary nucleic acid sequence, e.g. U may be substituted by T.

Further, the invention encompasses nucleotide sequences which hybridize under stringent conditions with the nucleotide sequence as shown in Table 1, Table 2, Table 3 or Table 4, a complementary sequence thereof or a highly identical sequence. Stringent hybridization conditions comprise washing for 1 h in 1 x SSC and 0.1% SDS at 45°C, preferably at 48°C and more preferably at 50°C, particularly for 1 h in 0.2 x SSC and 0.1% SDS.

The isolated nucleic acid molecules of the invention preferably have a length of from 18 to 100 nucleotides, and more preferably from 18 to 80 nucleotides. It should be noted that mature miRNAs usually have a length of 19-24 nucleotides, particularly 21, 22 or 23 nucleotides. The miRNAs, however, may be also provided as a precursor which usually has a length of 50-90 nucleotides, particularly 60-80 nucleotides. It should be noted that the precursor may be produced by processing of a primary transcript which may have a length of >100 nucleotides.

The nucleic acid molecules may be present in single-stranded or double-stranded form. The miRNA as such is usually a single-stranded molecule, while the mi-precursor is usually an at least partially self-complementary molecule capable of forming double-stranded portions, e.g. stem- and loop-structures. DNA molecules encoding the miRNA and miRNA precursor molecules. The nucleic acids may be selected from RNA, DNA or nucleic acid analog molecules, such as sugar- or backbone-modified ribonucleotides or deoxyribonucleotides. It should be noted, however, that other nucleic analogs, such as peptide nucleic acids (PNA) or locked nucleic acids (LNA), are also suitable.

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In an embodiment of the invention the nucleic acid molecule is an RNA- or DNA molecule, which contains at least one modified nucleotide analog, i.e. a naturally occurring ribonucleotide or deoxyribonucleotide is substituted by a non-naturally occurring nucleotide. The modified nucleotide analog may be located for example at the 5'-end and/or the 3'-end of the nucleic acid molecule.

Preferred nucleotide analogs are selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, i.e. ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, e.g. 5-(2-amino)propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, e.g. 8-bromo guanosine; deaza nucleotides, e.g. 7-deaza-adenosine; O- and N-alkylated nucleotides, e.g. N6-methyl adenosine are suitable. In preferred sugar-modified ribonucleotides the 2'-OH-group is replaced by a group selected from H, OR, R, halo, SH, SR, NH₂, NHR, NR₂ or CN, wherein R is C₁-C₈ alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. In preferred backbone-modified ribonucleotides the phosphoester group connecting to adjacent ribonucleotides is replaced by a modified group, e.g. of phosphothioate group. It should be noted that the above modifications may be combined.

The nucleic acid molecules of the invention may be obtained by chemical synthesis methods or by recombinant methods, e.g. by enzymatic transcription from synthetic DNA-templates or from DNA-plasmids isolated from recombinant organisms. Typically phage RNA-polymerases are used for transcription, such as T7, T3 or SP6 RNA-polymerases.

The invention also relates to a recombinant expression vector comprising a recombinant nucleic acid operatively linked to an expression control sequence, wherein expression, i.e. transcription and optionally further

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processing results in a miRNA-molecule or miRNA precursor molecule as described above. The vector is preferably a DNA-vector, e.g. a viral vector or a plasmid, particularly an expression vector suitable for nucleic acid expression in eukaryotic, more particularly mammalian cells. The recombinant nucleic acid contained in said vector may be a sequence which results in the transcription of the miRNA-molecule as such, a precursor or a primary transcript thereof, which may be further processed to give the miRNA-molecule.

Further, the invention relates to diagnostic or therapeutic applications of the claimed nucleic acid molecules. For example, miRNAs may be detected in biological samples, e.g. in tissue sections, in order to determine and classify certain cell types or tissue types or miRNA-associated pathogenic disorders which are characterized by differential expression of miRNA-molecules or miRNA-molecule patterns. Further, the developmental stage of cells may be classified by determining temporarily expressed miRNA-molecules.

Further, the claimed nucleic acid molecules are suitable for therapeutic applications. For example, the nucleic acid molecules may be used as modulators or targets of developmental processes or disorders associated with developmental dysfunctions, such as cancer. For example, miR-15 and miR-16 probably function as tumor-suppressors and thus expression or delivery of these RNAs or analogs or precursors thereof to tumor cells may provide therapeutic efficacy, particularly against leukemias, such as B-cell chronic lymphocytic leukemia (B-CLL). Further, miR-10 is a possible regulator of the translation of Hox Genes, particularly Hox 3 and Hox 4 (or Scr and Dfd in *Drosophila*).

In general, the claimed nucleic acid molecules may be used as a modulator of the expression of genes which are at least partially complementary to said nucleic acid. Further, miRNA molecules may act as target for

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therapeutic screening procedures, e.g. inhibition or activation of miRNA molecules might modulate a cellular differentiation process, e.g. apoptosis.

Furthermore, existing miRNA molecules may be used as starting materials
5 for the manufacture of sequence-modified miRNA molecules, in order to modify the target-specificity thereof, e.g. an oncogene, a multidrug-resistance gene or another therapeutic target gene. The novel engineered miRNA molecules preferably have an identity of at least 80% to the starting miRNA, e.g. as depicted in Tables 1, 2, 3 and 4. Further, miRNA
10 molecules can be modified, in order that they are symmetrically processed and then generated as double-stranded siRNAs which are again directed against therapeutically relevant targets.

Furthermore, miRNA molecules may be used for tissue reprogramming
15 procedures, e.g. a differentiated cell line might be transformed by expression of miRNA molecules into a different cell type or a stem cell.

For diagnostic or therapeutic applications, the claimed RNA molecules are preferably provided as a pharmaceutical composition. This pharmaceutical
20 composition comprises as an active agent at least one nucleic acid molecule as described above and optionally a pharmaceutically acceptable carrier.

The administration of the pharmaceutical composition may be carried out
25 by known methods, wherein a nucleic acid is introduced into a desired target cell in vitro or in vivo.

Commonly used gene transfer techniques include calcium phosphate, DEAE-dextran, electroporation and microinjection and viral methods [30,
30 31, 32, 33, 34]. A recent addition to this arsenal of techniques for the introduction of DNA into cells is the use of cationic liposomes [35].

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Commercially available cationic lipid formulations are e.g. Tfx 50 (Promega) or Lipofectamin 2000 (Life Technologies).

The composition may be in form of a solution, e.g. an injectable solution, a cream, ointment, tablet, suspension or the like. The composition may be administered in any suitable way, e.g. by injection, by oral, topical, nasal, rectal application etc. The carrier may be any suitable pharmaceutical carrier. Preferably, a carrier is used, which is capable of increasing the efficacy of the RNA molecules to enter the target-cells. Suitable examples of such carriers are liposomes, particularly cationic liposomes.

Further, the invention relates to a method of identifying novel microRNA-molecules and precursors thereof, in eukaryotes, particularly in vertebrates and more particularly in mammals, such as humans or mice. This method comprises: ligating 5'- and 3'-adapter-molecules to the end of a size-fractionated RNA-population, reverse transcribing said adapter-ligated RNA-population, and characterizing said reverse transcribed RNA-molecules, e.g. by amplification, concatamerization, cloning and sequencing.

A method as described above already has been described in (8), however, for the identification of siRNA molecules. Surprisingly, it was found now that the method is also suitable for identifying the miRNA molecules or precursors thereof as claimed in the present application.

Further, it should be noted that as 3'-adaptor for derivatization of the 3'-OH group not only 4-hydroxymethylbenzyl but other types of derivatization groups, such as alkyl, alkyl amino, ethylene glycol or 3'-deoxy groups are suitable.

Further, the invention shall be explained in more detail by the following Figures and Examples:

Figure Legends

Fig. 1A. Expression of *D. melanogaster* miRNAs. Northern blots of total RNA isolated from staged populations of *D. melanogaster* were probed for the indicated miRNAs. The position of 76-nt val-tRNA is also indicated on the blots. 5S rRNA serves as loading control. E, embryo; L, larval stage; P, pupae; A, adult; S2, Schneider-2 cells. It should be pointed out, that S2 cells are polyclonal, derived from an unknown subset of embryonic tissues, and may have also lost some features of their tissue of origin while maintained in culture. miR-3 to miR-6 RNAs were not detectable in S2 cells (data not shown). miR-14 was not detected by Northern blotting and may be very weakly expressed, which is consistent with its cloning frequency. Similar miRNA sequences are difficult to distinguish by Northern blotting because of potential cross-hybridization of probes.

Fig. 1B. Expression of vertebrate miRNAs. Northern blots of total RNA isolated from HeLa cells, mouse kidneys, adult zebrafish, frog ovaries, and S2 cells were probed for the indicated miRNAs. The position of 76-nt val-tRNA is also indicated on the blots. 5S rRNA from the preparations of total RNA from the indicated species is also shown. The gels used for probing of miR-18, miR-19a, miR-30, and miR-31 were not run as far as the other gels (see tRNA marker position). miR-32 and miR-33 were not detected by Northern blotting, which is consistent with their low cloning frequency. Oligodeoxynucleotides used as Northern probes were:

let-7a, 5' TACTATACAACCTACTACCTCAATTTGCC (SEQ ID NO:1);

let-7d, 5' ACTATGCAACCTACTACCTCT (SEQ ID NO:2);

let-7e, 5' ACTATACAACCTCCTACCTCA (SEQ ID NO:3);

D. melanogaster val-tRNA, 5' TGGTGTTCGCCCCGGGAA (SEQ ID NO:4);

miR-1, 5' TGGAATGTAAAGAAGTATGGAG (SEQ ID NO:5);

miR-2b, 5' GCTCCTCAAAGCTGGCTGTGATA (SEQ ID NO:6);

miR-3, 5' TGAGACACACTTTGCCAGTGA (SEQ ID NO:7);

miR-4, 5' TCAATGGTTGTCTAGCTTTAT (SEQ ID NO:8);

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miR-5, 5' CATATCACAACGATCGTTCCTTT (SEQ ID NO:9);
 miR-6, 5' AAAAAGAACAGCCACTGTGATA (SEQ ID NO:10);
 miR-7, 5' TGGAAGACTAGTGATTTTGTGTTGT (SEQ ID NO:11);
 miR-8, 5' GACATCTTTACCTGACAGTATTA (SEQ ID NO:12);
 5 miR-9, 5' TCATACAGCTAGATAACCAAAGA (SEQ ID NO:13);
 miR-10, 5' ACAAATTCGGATCTACAGGGT (SEQ ID NO:14);
 miR-11, 5' GCAAGAACTCAGACTGTGATG (SEQ ID NO:15);
 miR-12, 5' ACCAGTACCTGATGTAATACTCA (SEQ ID NO:16);
 miR-13a, 5' ACTCGTCAAATGGCTGTGATA (SEQ ID NO:17);
 10 miR-14, 5' TAGGAGAGAGAAAAAGACTGA (SEQ ID NO:18);
 miR-15, 5' TAGCAGCACATAATGGTTTGT (SEQ ID NO:19);
 miR-16, 5' GCCAATATTTACGTGCTGCTA (SEQ ID NO:20);
 miR-17, 5' TACAAGTGCCTTCACTGCAGTA (SEQ ID NO:21);
 miR-18, 5' TATCTGCACTAGATGCACCTTA (SEQ ID NO:22);
 15 miR-19a, 5' TCAGTTTTGCATAGATTGACACA (SEQ ID NO:23);
 miR-20, 5' TACCTGCACTATAAGCACTTTA (SEQ ID NO:24);
 miR-21, 5' TCAACATCAGTCTGATAAGCTA (SEQ ID NO:25);
 miR-22, 5' ACAGTTCTTCAACTGGCAGCTT (SEQ ID NO:26);
 miR-23, 5' GGAAATCCCTGGCAATGTGAT (SEQ ID NO:27);
 20 miR-24, 5' CTGTTCTGCTGAACTGAGCCA (SEQ ID NO:28);
 miR-25, 5' TCAGACCGAGACAAGTGCAATG (SEQ ID NO:29);
 miR-26a, 5' AGCCTATCCTGGATTACTTGAA (SEQ ID NO:30);
 miR-27, 5' AGCGGAACTTAGCCACTGTGAA (SEQ ID NO:31);
 miR-28, 5' CTCAATAGACTGTGAGCTCCTT (SEQ ID NO:32);
 25 miR-29, 5' AACCGATTTCAGATGGTGCTAG (SEQ ID NO:33);
 miR-30, 5' GCTGCAAACATCCGACTGAAAG (SEQ ID NO:34);
 miR-31, 5' CAGCTATGCCAGCATCTTGCCT (SEQ ID NO:35);
 miR-32, 5' GCAACTTAGTAATGTGCAATA (SEQ ID NO:36);
 miR-33, 5' TGCAATGCAACTACAATGCACC (SEQ ID NO:37).

30

Fig. 2. Genomic organization of miRNA gene clusters. The precursor structure is indicated as box and the location of the miRNA within the

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precursor is shown in gray; the chromosomal location is also indicated to the right. (A) *D. melanogaster* miRNA gene clusters. (B) Human miRNA gene clusters. The cluster of let-7a-1 and let-7f-1 is separated by 26500 nt from a copy of let-7d on chromosome 9 and 17. A cluster of let-7a-3 and let-7b, separated by 938 nt on chromosome 22, is not illustrated.

Fig. 3. Predicted precursor structures of *D. melanogaster* miRNAs. RNA secondary structure prediction was performed using mfold version 3.1 [28] and manually refined to accommodate G/U wobble base pairs in the helical segments. The miRNA sequence is underlined. The actual size of the stem-loop structure is not known experimentally and may be slightly shorter or longer than represented. Multicopy miRNAs and their corresponding precursor structures are also shown.

Fig. 4. Predicted precursor structures of human miRNAs. For legend, see Fig. 3.

Fig. 5. Expression of novel mouse miRNAs. Northern blot analysis of novel mouse miRNAs. Total RNA from different mouse tissues was blotted and probed with a 5'-radiolabeled oligodeoxynucleotide complementary to the indicated miRNA. Equal loading of total RNA on the gel was verified by ethidium bromide staining prior to transfer; the band representing tRNAs is shown. The fold-back precursors are indicated with capital L. Mouse brains were dissected into midbrain, mb, cortex, cx, cerebellum, cb. The rest of the brain, rb, was also used. Other tissues were heart, ht, lung, lg, liver, lv, colon, co, small intestine, si, pancreas, pc, spleen, sp, kidney, kd, skeletal muscle, sm, stomach, st, H, human Hela SS3 cells. Oligodeoxynucleotides used as Northern probes were:

miR-1a, CTCCATACTTCTTTACATTCCA (SEQ ID NO:38);
 miR-30b, GCTGAGTGTAGGATGTTTACA (SEQ ID NO:39);
 miR-30a-s, GCTTCCAGTCGAGGATGTTTACA (SEQ ID NO:40);
 miR-99b, CGCAAGGTCGGTTCTACGGGTG (SEQ ID NO:41);

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miR-101, TCAGTTATCACAGTACTGTA (SEQ ID NO:42);
 miR-122a, ACAAACACCATTGTCACTCCA (SEQ ID NO:43);
 miR-124a, TGGCATTACCGCGTGCCTTA (SEQ ID NO:44);
 miR-125a, CACAGGTAAAGGGTCTCAGGGA (SEQ ID NO:45);
 5 miR-125b, TCACAAGTTAGGGTCTCAGGGA (SEQ ID NO:46);
 miR-127, AGCCAAGCTCAGACGGATCCGA (SEQ ID NO:47);
 miR-128, AAAAGAGACCGGTTCACTCTGA (SEQ ID NO:48);
 miR-129, GCAAGCCCAGACCGAAAAAAG (SEQ ID NO:49);
 miR-130, GCCCTTTTAACATTGCACTC (SEQ ID NO:50);
 10 miR-131, ACTTTCGGTTATCTAGCTTTA (SEQ ID NO:51);
 miR-132, ACGACCATGGCTGTAGACTGTTA (SEQ ID NO:52);
 miR-143, TGAGCTACAGTGCTTCATCTCA (SEQ ID NO:53).

15 Fig.6. Potential orthologs of lin-4 stRNA. (A) Sequence alignment of *C. elegans* lin-4 stRNA with mouse miR-125a and miR-125b and the *D. melanogaster* miR-125. Differences are highlighted by gray boxes. (B) Northern blot of total RNA isolated from staged populations of *D. melanogaster*, probed for miR-125. E, embryo; L, larval stage; P, pupae; A, adult; S2, Schneider-2 cells.
 20

Fig. 7. Predicted precursor structures of miRNAs, sequence accession numbers and homology information. RNA secondary structure prediction was performed using mfold version 3.1 and manually refined to
 25 accommodate G/U wobble base pairs in the helical segments. Dashes were inserted into the secondary structure presentation when asymmetrically bulged nucleotides had to be accommodated. The excised miRNA sequence is underlined. The actual size of the stem-loop structure is not known experimentally and may be slightly shorter or longer than
 30 represented. Multicopy miRNAs and their corresponding precursor structures are also shown. In cases where no mouse precursors were yet deposited in the database, the human orthologs are indicated. miRNAs

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which correspond to *D. melanogaster* or human sequences are included. Published *C. elegans* miRNAs [36, 37] are also included in the table. A recent set of new HeLa cell miRNAs is also indicated [46]. If several ESTs were retrieved for one organism in the database, only those with different precursor sequences are listed. miRNA homologs found in other species are indicated. Chromosomal location and sequence accession numbers, and clusters of miRNA genes are indicated. Sequences from cloned miRNAs were searched against mouse and human in GenBank (including trace data), and against *Fugu rubripes* and *Danio rerio* at www.jgi.doe.gov and www.sanger.ac.uk, respectively.

EXAMPLE 1: MicroRNAs from *D. melanogaster* and human.

We previously developed a directional cloning procedure to isolate siRNAs after processing of long dsRNAs in *Drosophila melanogaster* embryo lysate (8). Briefly, 5' and 3' adapter molecules were ligated to the ends of a size-fractionated RNA population, followed by reverse transcription, PCR amplification, concatamerization, cloning and sequencing. This method, originally intended to isolate siRNAs, led to the simultaneous identification of 14 novel 20- to 23-nt short RNAs which are encoded in the *D. melanogaster* genome and which are expressed in 0 to 2 h embryos (Table 1). The method was adapted to clone RNAs in a similar size range from HeLa cell total RNA (14), which led to the identification of 19 novel human stRNAs (Table 2), thus providing further evidence for the existence of a large class of small RNAs with potential regulatory roles. According to their small size, we refer to these novel RNAs as microRNAs or miRNAs. The miRNAs are abbreviated as miR-1 to miR-33, and the genes encoding miRNAs are named mir-1 to mir-33. Highly homologous miRNAs are classified by adding a lowercase letter, followed by a dash and a number for designating multiple genomic copies of a mir gene.

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The expression and size of the cloned, endogenous short RNAs was also examined by Northern blotting (Fig. 1, Table 1 and 2). Total RNA isolation was performed by acid guanidinium thiocyanate-phenol-chloroform extraction [45]. Northern analysis was performed as described [1], except that the total RNA was resolved on a 15% denaturing polyacrylamide gel, transferred onto Hybond-N+ membrane (Amersham Pharmacia Biotech), and the hybridization and wash steps were performed at 50°C. Oligodeoxynucleotides used as Northern probes were 5'-32P-phosphorylated, complementary to the miRNA sequence and 20 to 25 nt in length.

5S rRNA was detected by ethidium staining of polyacrylamide gels prior to transfer. Blots were stripped by boiling in 0.1% aqueous sodium dodecylsulfate/0.1x SSC (15 mM sodium chloride, 1.5 mM sodium citrate, pH 7.0) for 10 min, and were re-probed up to 4 times until the 21-nt signals became too weak for detection. Finally, blots were probed for val-tRNA as size marker.

For analysis of *D. melanogaster* RNAs, total RNA was prepared from different developmental stages, as well as cultured Schneider-2 (S2) cells, which originally derive from 20-24 h *D. melanogaster* embryos [15] (Fig. 1, Table 1). miR-3 to miR-7 are expressed only during embryogenesis and not at later developmental stages. The temporal expression of miR-1, miR-2 and miR-8 to miR-13 was less restricted. These miRNAs were observed at all developmental stages though significant variations in the expression levels were sometimes observed. Interestingly, miR-1, miR-3 to miR-6, and miR-8 to miR-11 were completely absent from cultured Schneider-2 (S2) cells, which were originally derived from 20-24 h *D. melanogaster* embryos [15], while miR-2, miR-7, miR-12, and miR-13 were present in S2 cells, therefore indicating cell type-specific miRNA expression. miR-1, miR-8, and miR-12 expression patterns are similar to those of *lin-4* stRNA in *C. elegans*, as their expression is strongly upregulated in larvae and sustained

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to adulthood [16]. miR-9 and miR-11 are present at all stages but are strongly reduced in the adult which may reflect a maternal contribution from germ cells or expression in one sex only.

5 The mir-3 to mir-6 genes are clustered (Fig. 2A), and mir-6 is present as triple repeat with slight variations in the mir-6 precursor sequence but not in the miRNA sequence itself. The expression profiles of miR-3 to miR-6 are highly similar (Table 1), which suggests that a single embryo-specific precursor transcript may give rise to the different miRNAs, or that the
10 same enhancer regulates miRNA-specific promoters. Several other fly miRNAs are also found in gene clusters (Fig. 2A).

The expression of HeLa cell miR-15 to miR-33 was examined by Northern blotting using HeLa cell total RNA, in addition to total RNA prepared from
15 mouse kidneys, adult zebrafish, *Xenopus laevis* ovary, and *D. melanogaster* S2 cells (Fig. 1B, Table 2). miR-15 and miR-16 are encoded in a gene cluster (Fig. 2B) and are detected in mouse kidney, fish, and very weakly in frog ovary, which may result from miRNA expression in somatic ovary tissue rather than oocytes. mir-17 to mir-20 are also clustered (Fig. 2B),
20 and are expressed in HeLa cells and fish, but undetectable in mouse kidney and frog ovary (Fig. 1, Table 2), and therefore represent a likely case of tissue-specific miRNA expression.

The majority of vertebrate and invertebrate miRNAs identified in this study
25 are not related by sequence, but a few exceptions, similar to the highly conserved let-7 RNA [6], do exist. Sequence analysis of the *D. melanogaster* miRNAs revealed four such examples of sequence conservation between invertebrates and vertebrates. miR-1 homologs are encoded in the genomes of *C. elegans*, *C. briggsae*, and humans, and are
30 found in cDNAs from zebrafish, mouse, cow and human. The expression of mir-1 was detected by Northern blotting in total RNA from adult zebrafish and *C. elegans*, but not in total RNA from HeLa cells or mouse kidney

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(Table 2 and data not shown). Interestingly, while mir-1 and let-7 are expressed both in adult flies (Fig. 1A) [6] and are both undetected in S2 cells, miR-1 is, in contrast to let-7, undetectable in HeLa cells. This represents another case of tissue-specific expression of a miRNA, and indicates that miRNAs may not only play a regulatory role in developmental timing, but also in tissue specification. miR-7 homologs were found by database searches in mouse and human genomic and expressed sequence tag sequences (ESTs). Two mammalian miR-7 variants are predicted by sequence analysis in mouse and human, and were detected by Northern blotting in HeLa cells and fish, but not in mouse kidney (Table 2). Similarly, we identified mouse and human miR-9 and miR-10 homologs by database searches but only detected mir-10 expression in mouse kidney.

The identification of evolutionary related miRNAs, which have already acquired multiple sequence mutations, was not possible by standard bioinformatic searches. Direct comparison of the *D. melanogaster* miRNAs with the human miRNAs identified an 11-nt segment shared between *D. melanogaster* miR-6 and HeLa miR-27, but no further relationships were detected. One may speculate that most miRNAs only act on a single target and therefore allow for rapid evolution by covariation, and that highly conserved miRNAs act on more than one target sequence, and therefore have a reduced probability for evolutionary drift by covariation [6]. An alternative interpretation is that the sets of miRNAs from *D. melanogaster* and humans are fairly incomplete and that many more miRNAs remain to be discovered, which will provide the missing evolutionary links.

lin-4 and let-7 stRNAs were predicted to be excised from longer transcripts that contain approximately 30 base-pair stem-loop structures [1, 6]. Database searches for newly identified miRNAs revealed that all miRNAs are flanked by sequences that have the potential to form stable stem-loop structures (Fig. 3 and 4). In many cases, we were able to detect the predicted, approximately 70-nt precursors by Northern blotting (Fig. 1).

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Some miRNA precursor sequences were also identified in mammalian cDNA (EST) databases [27], indicating that primary transcripts longer than 70-nt stem-loop precursors do also exist. We never cloned a 22-nt RNA complementary to any of the newly identified miRNAs, and it is as yet
5 unknown how the cellular processing machinery distinguishes between the miRNA and its complementary strand. Comparative analysis of the precursor stem-loop structures indicates that the loops adjacent to the base-paired miRNA segment can be located on either side of the miRNA sequence (Fig. 3 and 4), suggesting that the 5' or 3' location of the stem-
10 closing loop is not the determinant of miRNA excision. It is also unlikely that the structure, length or stability of the precursor stem is the critical determinant as the base-paired structures are frequently imperfect and interspersed by less stable, non-Watson-Crick base pairs such as G/A, U/U, C/U, A/A, and G/U wobbles. Therefore, a sequence-specific recognition
15 process is a likely determinant for miRNA excision, perhaps mediated by members of the Argonaute (rde-1/ago1/piwi) protein family. Two members of this family, alg-1 and alg-2, have recently been shown to be critical for stRNA processing in *C. elegans* [13]. Members of the Argonaute protein family are also involved in RNAi and PTGS. In *D. melanogaster*, these
20 include argonaute2, a component of the siRNA-endonuclease complex (RISC) [17], and its relative aubergine, which is important for silencing of repeat genes [18]. In other species, these include rde-1, argonaute1, and qde-2, in *C. elegans* [19], *Arabidopsis thaliana* [20], and *Neurospora crassa* [21], respectively. The Argonaute protein family therefore represents,
25 besides the RNase III Dicer [12, 13], another evolutionary link between RNAi and miRNA maturation.

Despite advanced genome projects, computer-assisted detection of genes encoding functional RNAs remains problematic [22]. Cloning of expressed,
30 short functional RNAs, similar to EST approaches (RNomics), is a powerful alternative and probably the most efficient method for identification of such novel gene products [23-26]. The number of functional RNAs has been

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widely underestimated and is expected to grow rapidly because of the development of new functional RNA cloning methodologies.

The challenge for the future is to define the function and the potential targets of these novel miRNAs by using bioinformatics as well as genetics, and to establish a complete catalogue of time- and tissue-specific distribution of the already identified and yet to be uncovered miRNAs. lin-4 and let-7 stRNAs negatively regulate the expression of proteins encoded by mRNAs whose 3' untranslated regions contain sites of complementarity to the stRNA [3-5].

Thus, a series of 33 novel genes, coding for 19- to 23-nucleotide microRNAs (miRNAs), has been cloned from fly embryos and human cells. Some of these miRNAs are highly conserved between vertebrates and invertebrates and are developmentally or tissue-specifically expressed. Two of the characterized human miRNAs may function as tumor suppressors in B-cell chronic lymphocytic leukemia. miRNAs are related to a small class of previously described 21- and 22-nt RNAs (lin-4 and let-7 RNAs), so-called small temporal RNAs (stRNAs), and regulate developmental timing in *C. elegans* and other species. Similar to stRNAs, miRNAs are presumed to regulate translation of specific target mRNAs by binding to partially complementary sites, which are present in their 3'-untranslated regions.

Deregulation of miRNA expression may be a cause of human disease, and detection of expression of miRNAs may become useful as a diagnostic. Regulated expression of miRNAs in cells or tissue devoid of particular miRNAs may be useful for tissue engineering, and delivery or transgenic expression of miRNAs may be useful for therapeutic intervention. miRNAs may also represent valuable drug targets itself. Finally, miRNAs and their precursor sequences may be engineered to recognize therapeutic valuable targets.

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EXAMPLE 2: miRNAs from mouse.

To gain more detailed insights into the distribution and function of miRNAs in mammals, we investigated the tissue-specific distribution of miRNAs in adult mouse. Cloning of miRNAs from specific tissues was preferred over whole organism-based cloning because low-abundance miRNAs that normally go undetected by Northern blot analysis are identified clonally. Also, in situ hybridization techniques for detecting 21-nt RNAs have not yet been developed. Therefore, 19- to 25-nucleotide RNAs were cloned and sequenced from total RNA, which was isolated from 18.5-weeks old BL6 mice. Cloning of miRNAs was performed as follows: 0.2 to 1 mg of total RNA was separated on a 15% denaturing polyacrylamide gel and RNA of 19- to 25-nt size was recovered. A 5'-phosphorylated 3'-adapter oligonucleotide (5'-pUUUaaccgcgaattccagx: uppercase, RNA; lowercase, DNA; p, phosphate; x, 3'-Amino-Modifier C-7, ChemGenes, Ashland, Ma, USA, Cat. No. NSS-1004; SEQ ID NO:54) and a 5'-adapter oligonucleotide (5'-acggaattcctcactAAA: uppercase, RNA; lowercase, DNA; SEQ ID NO:55) were ligated to the short RNAs. RT/PCR was performed with 3'-primer (5'-GACTAGCTGGAATTCGCGGTAAA; SEQ ID NO:56) and 5'-primer (5'-CAGCCAACGGAATTCCTCACTAAA; SEQ ID NO:57). In order to introduce Ban I restriction sites, a second PCR was performed using the primer pair 5'-CAGCCAACAGGCACCGAATTCCTCACTAAA (SEQ ID NO:57) and 5'-GACTAGCTTGGTGCCGAATTCGCGGTAAA (SEQ ID NO:56), followed by concatamerization after Ban I digestion and T4 DNA ligation. Concatamers of 400 to 600 basepairs were cut out from 1.5% agarose gels and recovered by Biotrap (Schleicher & Schuell) electroelution (1x TAE buffer) and by ethanol precipitation. Subsequently, the 3' ends of the concatamers were filled in by incubating for 15 min at 72°C with Taq polymerase in standard PCR reaction mixture. This solution was diluted 3-fold with water and directly used for ligation into pCR2.1 TOPO vectors. Clones were screened for inserts by PCR and 30 to 50 samples were subjected to sequencing. Because RNA was prepared from combining

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tissues of several mice, minor sequence variations that were detected multiple times in multiple clones may reflect polymorphisms rather than RT/PCR mutations. Public database searching was used to identify the genomic sequences encoding the approx. 21-nt RNAs. The occurrence of
5 a 20 to 30 basepair fold-back structure involving the immediate upstream or downstream flanking sequences was used to assign miRNAs [36-38].

We examined 9 different mouse tissues and identified 34 novel miRNAs, some of which are highly tissue-specifically expressed (Table 3 and Figure
10 5). Furthermore, we identified 33 new miRNAs from different mouse tissues and also from human Soas-2 osteosarcoma cells (Table 4). miR-1 was previously shown by Northern analysis to be strongly expressed in adult heart, but not in brain, liver, kidney, lung or colon [37]. Here we show that miR-1 accounts for 45% of all mouse miRNAs found in heart, yet miR-1 was still expressed at a low level in liver and midbrain even
15 though it remained undetectable by Northern analysis. Three copies or polymorphic alleles of miR-1 were found in mice. The conservation of tissue-specific miR-1 expression between mouse and human provides additional evidence for a conserved regulatory role of this miRNA. In liver, variants of miR-122 account for 72% of all cloned miRNAs and miR-122
20 was undetected in all other tissues analyzed. In spleen, miR-143 appeared to be most abundant, at a frequency of approx. 30%. In colon, miR-142-as, was cloned several times and also appeared at a frequency of 30%. In small intestine, too few miRNA sequences were obtained to permit
25 statistical analysis. This was due to strong RNase activity in this tissue, which caused significant breakdown of abundant non-coding RNAs, e.g. rRNA, so that the fraction of miRNA in the cloned sequences was very low. For the same reason, no miRNA sequences were obtained from
pancreas.

30 To gain insights in neural tissue miRNA distribution, we analyzed cortex, cerebellum and midbrain. Similar to heart, liver and small intestine, variants

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of a particular miRNA, miR-124, dominated and accounted for 25 to 48% of all brain miRNAs. miR-101, -127, -128, -131, and -132, also cloned from brain tissues, were further analyzed by Northern blotting and shown to be predominantly brain-specific. Northern blot analysis was performed as described in Example 1. tRNAs and 5S rRNA were detected by ethidium staining of polyacrylamide gels prior to transfer to verify equal loading. Blots were stripped by boiling in deionized water for 5 min, and reprobed up to 4 times until the 21-nt signals became too weak for detection.

miR-125a and miR-125b are very similar to the sequence of *C. elegans* lin-4 stRNA and may represent its orthologs (Fig. 6A). This is of great interest because, unlike let-7 that was readily detected in other species, lin-4 has acquired a few mutations in the central region and thus escaped bioinformatic database searches. Using the mouse sequence miR-125b, we could readily identify its ortholog in the *D. melanogaster* genome. miR-125a and miR-125b differ only by a central diuridine insertion and a U to C change. miR-125b is very similar to lin-4 stRNA with the differences located only in the central region, which is presumed to be bulged out during target mRNA recognition [41]. miR-125a and miR-125b were cloned from brain tissue, but expression was also detected by Northern analysis in other tissues, consistent with the role for lin-4 in regulating neuronal remodeling by controlling lin-14 expression [43]. Unfortunately, orthologs to *C. elegans* lin-14 have not been described and miR-125 targets remain to be identified in *D. melanogaster* or mammals. Finally, miR-125b expression is also developmentally regulated and only detectable in pupae and adult but not in embryo or larvae of *D. melanogaster* (Fig. 6B).

Sequence comparison of mouse miRNAs with previously described miRNA reveals that miR-99b and miR-99a are similar to *D. melanogaster*, mouse and human miR-10 as well as *C. elegans* miR-51 [36], miR-141 is similar to *D. melanogaster* miR-8, miR-29b is similar to *C. elegans* miR-83, and miR-131 and miR-142-s are similar to *D. melanogaster* miR-4 and *C.*

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elegans miR-79 [36]. miR-124a is conserved between invertebrates and vertebrates. In this respect it should be noted that for almost every miRNA cloned from mouse was also encoded in the human genome, and frequently detected in other vertebrates, such as the pufferfish, *Fugu rubripes*, and the zebrafish, *Danio rerio*. Sequence conservation may point to conservation in function of these miRNAs. Comprehensive information about orthologous sequences is listed in Fig. 7.

In two cases both strands of miRNA precursors were cloned (Table 3), which was previously observed once for a *C. elegans* miRNA [36]. It is thought that the most frequently cloned strand of a miRNA precursor represents the functional miRNA, which is miR-30c-s and miR-142-as, s and as indicating the 5' or 3' side of the fold-back structure, respectively.

The mir-142 gene is located on chromosome 17, but was also found at the breakpoint junction of a t(8;17) translocation, which causes an aggressive B-cell leukemia due to strong up-regulation of a translocated MYC gene [44]. The translocated MYC gene, which was also truncated at the first exon, was located only 4-nt downstream of the 3'-end of the miR-142 precursor. This suggests that translocated MYC was under the control of the upstream miR-142 promoter. Alignment of mouse and human miR-142 containing EST sequences indicate an approximately 20 nt conserved sequence element downstream of the mir-142 hairpin. This element was lost in the translocation. It is conceivable that the absence of the conserved downstream sequence element in the putative miR-142/mRNA fusion prevented the recognition of the transcript as a miRNA precursor and therefore may have caused accumulation of fusion transcripts and overexpression of MYC.

miR-155, which was cloned from colon, is excised from the known noncoding BIC RNA [47]. BIC was originally identified as a gene transcriptionally activated by promoter insertion at a common retroviral

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integration site in B cell lymphomas induced by avian leukosis virus. Comparison of BIC cDNAs from human, mouse and chicken revealed 78% identity over 138 nucleotides [47]. The identity region covers the miR-155 fold-back precursor and a few conserved boxes downstream of the fold-back sequence. The relatively high level of expression of BIC in lymphoid organs and cells in human, mouse and chicken implies an evolutionary conserved function, but BIC RNA has also been detected at low levels in non-hematopoietic tissues [47].

Another interesting observation was that segments of perfect complementarity to miRNAs are not observed in mRNA sequences or in genomic sequences outside the miRNA inverted repeat. Although this could be fortuitous, based on the link between RNAi and miRNA processing [11, 13, 43] it may be speculated that miRNAs retain the potential to cleave perfectly complementary target RNAs. Because translational control without target degradation could provide more flexibility it may be preferred over mRNA degradation.

In summary, 63 novel miRNAs were identified from mouse and 4 novel miRNAs were identified from human Soas-2 osteosarcoma cells (Table 3 and Table 4), which are conserved in human and often also in other non-mammalian vertebrates. A few of these miRNAs appear to be extremely tissue-specific, suggesting a critical role for some miRNAs in tissue-specification and cell lineage decisions. We may have also identified the fruitfly and mammalian ortholog of *C. elegans* lin-4 stRNA. The establishment of a comprehensive list of miRNA sequences will be instrumental for bioinformatic approaches that make use of completed genomes and the power of phylogenetic comparison in order to identify miRNA-regulated target mRNAs.

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ligation (8). After ligation of concatamers into pCR2.1 TOPO vectors, about 100 clones were selected and subjected to sequencing.

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Table 1

D. melanogaster miRNAs. The sequences given represent the most abundant, and typically longest miRNA sequence identified by cloning; miRNAs frequently vary in length by one or two nucleotides at their 3' termini. From 222 short RNAs sequenced, 69 (31%) corresponded to miRNAs, 103 (46%) to already characterized functional RNAs (rRNA, 7SL RNA, tRNAs), 30 (14%) to transposon RNA fragments, and 20 (10%) sequences with no database entry. The frequency (freq.) for cloning a particular miRNA relative to all identified miRNAs is indicated in percent.

Results of Northern blotting of total RNA isolated from staged populations of D. melanogaster are summarized. E, embryo; L, larval stage; P, pupae; A, adult; S2, Schneider-2 cells. The strength of the signal within each blot is represented from strongest (+ + +) to undetected (-). let-7 stRNA was probed as control. Genbank accession numbers and homologs of miRNAs identified by database searching in other species are provided as supplementary material.

miRNA	sequence (5' to 3')	freq. (%)	E 0-3 h	E 0-6 h	L1+ L2	L3	P	A	S2
miR-1	UGGAAUGUAAAGAAGUAUGGAG (SEQ ID NO:58)	32	+	+	++ +	++ +	++	++ +	-
miR-2a*	UAUCACAGCCAGCUUGAUGAGC (SEQ ID NO:59)	3							
miR-2b*	UAUCACAGCCAGCUUGAGGAGC (SEQ ID NO:60)	3	++	++	++	++ +	++	+	++ +
miR-3	UCACUGGGCAAAGUGUGUCUCA#	9	+++	+++	-	-	-	-	-
miR-4	AUAAAGCUAGACAACCAUUGA (SEQ ID NO:62)	6	+++	+++	-	-	-	-	-
miR-5	AAAGGAACGAUCGUUGUGAUAUG (SEQ ID NO:63)	1	+++	+++	+/-	+/-	-	-	-
miR-6	UAUCACAGUGGCUGUUCUUUUU (SEQ ID NO:64)	13	+++	+++	+/-	+/-	-	-	-
miR-7	UGGAAGACUAGUGAUUUUGUUGU (SEQ ID NO:65)	4	+++	++	+/-	+/-	+/-	+/-	+/-
miR-8	UAAUACUGUCAGGUAAAGAUGUC (SEQ ID NO:66)	3	+/-	+/-	++ +	++ +	+	++ +	-

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miR-9	UCUUUGGUUAUCUAGCUGUAUGA (SEQ ID NO: 67)	7	+++	++	++	++	++	++	+/-	-
miR-10	ACCCUGUAGAUCCGAAUUUGU (SEQ ID NO: 68)	1	+	+	++	++	++	++	+	-
miR-11	CAUCACAGUCUGAGUUCUUGC (SEQ ID NO: 69)	7	+++	+++	++	++	++	++	+	-
miR-12	UGAGUAUUACAUCAGGUACUGGU (SEQ ID NO: 70)	7	+	+	++	++	++	++	+	+/-
5 miR-13a*	UAUCACAGCCAUUUUGACGAGU (SEQ ID NO: 71)	1	+++	+++	++	++	++	++	++	++
miR-13b*	UAUCACAGCCAUUUUGAUGAGU (SEQ ID NO: 72)	0								
miR-14	UCAGUCUUUUUCUCUCUCCUA (SEQ ID NO: 73)	1	-	-	-	-	-	-	-	-
let-7	UGAGGUAGUAGGUUGUAUAGUU (SEQ ID NO: 74)	0	-	-	-	-	++	++	++	-

10 # = (SEQ ID NO:61)

*Similar miRNA sequences are difficult to distinguish by Northern blotting because of potential cross-hybridization of probes.

Table 2

Human miRNAs. From 220 short RNAs sequenced, 100 (45%) corresponded to miRNAs, 53 (24%) to already characterized functional RNAs (rRNA, snRNAs, tRNAs), and 67 (30%) sequences with no database entry. Results of Northern blotting of total RNA isolated from different vertebrate species and S2 cells are indicated. For legend, see Table 1.

miRNA	sequence (5' to 3')	freq. (%)	HeLa cells	mouse kidney	adult fish	frog ovary	S2
let-7a*	UGAGGUAGUAGGUUGUAUAGUU#	10	+++	+++	+++	-	-
let-7b*	UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:76)	13					
let-7c*	UGAGGUAGUAGGUUGUAUGGUU (SEQ ID NO:77)	3					
let-7d*	AGAGGUAGUAGGUUGCAUAGU (SEQ ID NO:78)	2	+++	+++	+++	-	-
let-7e*	UGAGGUAGGAGGUUGUAUAGU (SEQ ID NO:79)	2	+++	+++	+++	-	-
let-7f*	UGAGGUAGUAGAUUGUAUAGUU (SEQ ID NO:80)	1					
miR-15	UAGCAGCACAUAAUGGUUUGUG (SEQ ID NO:81)	3	+++	++	+	+/-	-
miR-16	UAGCAGCAGUAAAUAUUGGCG (SEQ ID NO:82)	10	+++	+	+/-	+/-	-
miR-17	ACUGCAGUGAAGGCACUUGU (SEQ ID NO:83)	1	+++	-	-	-	-
miR-18	UAAGGUGCAUCUAGUGCAGAU (SEQ ID NO:84)	2	+++	-	-	-	-
miR-19a*	UGUGCAAUUCUAUGCAAACUGA (SEQ ID NO:85)	1	+++	-	+/-	-	-
miR-19b*	UGUGCAAUCCAUGCAAACUGA (SEQ ID NO:86)	3					
miR-20	UAAAGUGCUUAUAGUGCAGGUA (SEQ ID NO:87)	4	+++	-	+	-	-
miR-21	UAGCUUAUCAGACUGAUGUUGA (SEQ ID NO:88)	10	+++	+	++	-	-
miR-22	AAGCUGCCAGUUGAAGAACUGU (SEQ ID NO:89)	10	+++	+++	+	+/-	-
miR-23	AUCACAUUGCCAGGGAUUUC (SEQ ID NO:90)	2	+++	+++	+++	+	-

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	miR-24	UGGCUAGUUCAGCAGGAACAG (SEQ ID NO:91)	4	++	+++	++	-	-
	miR-25	CAUUGCACUUGUCUCGGUCUGA (SEQ ID NO:92)	3	+++	+	++	-	-
	miR-26a*	UUCAAGUAAUCCAGGAUAGGCU (SEQ ID NO:93)	2	+	++	+++	-	-
	miR-26b*	UUCAAGUAAUCCAGGAUAGGUU (SEQ ID NO:94)	1					-
5	miR-27	UUCACAGUGGCUAAGUCCGCU (SEQ ID NO:95)	2	+++	+++	++	-	-
	miR-28	AAGGAGCUCACAGUCUAUUGAG (SEQ ID NO:96)	2	+++	+++	-	-	-
	miR-29	CUAGCACCAUCUGAAAUCGGUU (SEQ ID NO:97)	2	+	+++	+/-	-	-
	miR-30	CUUUCAGUCGGAUGUUUGCAGC (SEQ ID NO:98)	2	+++	+++	+++	-	-
	miR-31	GGCAAGAUGCUGGCAUAGCUG (SEQ ID NO:99)	2	+++	-	-	-	-
10	miR-32	UAUUGCACAUAUACUAAGUUGC (SEQ ID NO:100)	1	-	-	-	-	-
	miR-33	GUGCAUUGUAGUUGCAUUG (SEQ ID NO:101)	1	-	-	-	-	-
	miR-1	UGGAUGUAAAAGAAGUAUGGAG (SEQ ID NO:102)	0	-	-	+	-	-
	miR-7	UGGAAGACUAGUGAUUUUGUUGU (SEQ ID NO:103)	0	+	-	+/-	-	+/-
	miR-9	UCUUUGGUUAUCUAGCUGUAUGA (SEQ ID NO:104)	0	-	-	-	-	-
15	miR-10	ACCCUGUAGAUCGAAUUUGU (SEQ ID NO:105)	0	-	+	-	-	-

= (SEQ ID NO:75)

*Similar miRNA sequences are difficult to distinguish by Northern blotting because of potential cross-hybridization of probes.

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Table 3

Mouse miRNAs. The sequences indicated represent the longest miRNA sequences identified by cloning. The 3'-terminus of miRNAs is often truncated by one or two nucleotides. miRNAs that are more than 85% identical in sequence (i.e. share 18 out of 21 nucleotides) or contain 1- or 2-nucleotide internal deletions are referred to by the same gene number followed by a lowercase letter. Minor sequence variations between related miRNAs are generally found near the ends of the miRNA sequence and are thought to not compromise target RNA recognition. Minor sequence variations may also represent A to G and C to U changes, which are accommodated as G-U wobble base pairs during target recognition. miRNAs with the suffix -s or -as indicate RNAs derived from either the 5'-half or the 3'-half of a miRNA precursor. Mouse brains were dissected into midbrain, mb, cortex, cx, cerebellum, cb. The tissues analyzed were heart, ht; liver, lv; small intestine, si; colon, co; cortex, ct; cerebellum, cb; midbrain, mb.

miRNA	sequence (5' to 3')	Number of clones							
		ht	lv	sp	si	co	cx	cb	mb
let-7a	UGAGGUAGUAGGUUGUAUAGUU (SEQ ID NO:106)	3				1	1		7
let-7b	UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:107)	1	1					2	5
let-7c	UGAGGUAGUAGGUUGUAUGGUU (SEQ ID NO:108)	2					2	5	19
let-7d	AGAGGUAGUAGGUUGCAUAGU (SEQ ID NO:109)	2				2	2		2
let-7e	UGAGGUAGGAGGUUGUAUAGU (SEQ ID NO:110)			1					2
let-7f	UGAGGUAGUAGAUUGUAUAGUU (SEQ ID NO:111)			2				3	3
let-7g	UGAGGUAGUAGUUUGUACAGUA (SEQ ID NO:112)						1	1	2
let-7h	UGAGGUAGUAGUGUGUACAGUU (SEQ ID NO:113)						1	1	

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	let-7i	UGAGGUAGUAGUUUGUGCU (SEQ ID NO:114)				1	1		
	miR-1b	UGGAAUGUAAAGAAGUAUGUAA (SEQ ID NO:115)	4	2					1
	miR-1c	UGGAAUGUAAAGAAGUAUGUAC (SEQ ID NO:116)	7						
	miR-1d	UGGAAUGUAAAGAAGUAUGUAUU (SEQ ID NO:117)	16						1
5	miR-9	UCUUUGGUUAUCUAGCUGUAUGA (SEQ ID NO:118)				3	4		4
	miR-15a	UAGCAGCACAUAAUGGUUUGUG (SEQ ID NO:119)	1						2
	miR-15b	UAGCAGCACAUCAUGGUUUACA (SEQ ID NO:120)	1						
	miR-16	UAGCAGCACGUAAAUUUGGCG (SEQ ID NO:121)	1		1	2	1	2	3
	miR-18	UAAGGUGCAUCUAGUGCAGAU (SEQ ID NO:122)		1					
10	miR-19b	UGUGCAAUCCAUCCAAACUGA (SEQ ID NO:123)		1					
	miR-20	UAAAGUGCUUAUAGUGCAGGUAG (SEQ ID NO:124)				1			
	miR-21	UAGCUUAUCAGACUGAUGUGA (SEQ ID NO:125)	1		1	2	1		
	miR-22	AAGCUGCCAGUUGAAGACUGU (SEQ ID NO:126)	2	1		1		1	2
	miR-23a	AUCACAUUGCCAGGGAUUUCC (SEQ ID NO:127)	1						
15	miR-23b	AUCACAUUGCCAGGGAUUACCAC (SEQ ID NO:128)					1		
	miR-24	UGGCUCAGUUCAGCAGGAACAG (SEQ ID NO:129)	1			1	1		1
	miR-26a	UUCAAGUAAUCCAGGAUAGGCU (SEQ ID NO:130)						3	2
	miR-26b	UUCAAGUAAUCCAGGAUAGGUU (SEQ ID NO:131)		2			4	1	
	miR-27a	UUCACAGUGGCUAAGUUCGCU (SEQ ID NO:132)	1		2		1	1	2
20	miR-27b	UUCACAGUGGCUAAGUUCUG (SEQ ID NO:133)							1
	miR-29a	CUAGCACCAUCUGAAUCCGUU (SEQ ID NO:134)	1			1		1	
	miR-29b/miR-102	UAGCACCAUUUGAAUCCAGUGUU (SEQ ID NO:135)	1			1	5		3
	miR-29c/	UAGCACCAUUUGAAUCCGUUA (SEQ ID NO:136)	1				3		1

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	miR-30a-s/miR-97	UGUAAACAUCCTUCGACUGGAAGC (SEQ ID NO:137)	1	1	1
	miR-30a-as ^a	CUUUCAGUCGGAUGUUUCAGC (SEQ ID NO:138)		1	
	miR-30b	UGUAAACAUCUACACUCAGC (SEQ ID NO:139)	1	2	
	miR-30c	UGUAAACAUCUACACUCUCAGC (SEQ ID NO:140)	2	1	1
5	miR-30d	UGUAAACAUCCTCGACUGGAAG (SEQ ID NO:141)	1		
	miR-99a/miR-99	ACCCGUAGAUCGUAUCUUGU (SEQ ID NO:142)		1	
	miR-99b	CACCCGUAGAACCGACCUUGCG (SEQ ID NO:143)		1	
	miR-101	UACAGUACUGUUAUACUGA (SEQ ID NO:144)		2	1 1
	miR-122a	UGGAGUGUGACAAUGGUGUUUGU (SEQ ID NO:145)	3		
10	miR-122b	UGGAGUGUGACAAUGGUGUUUGA (SEQ ID NO:146)	11		
	miR-122a,b	UGGAGUGUGACAAUGGUGUUUG (SEQ ID NO:147)	23		
	miR-123	CAUUAUUACUUUUGUACGCG (SEQ ID NO:148)	1 2		
	miR-124a ^b	UUAAGGCACGCGG-UGAAUGCCA (SEQ ID NO:149)	1	37	41 24
	miR-124b	UUAAGGCACGCGGGUGAAUGC (SEQ ID NO:150)		1	3
15	miR-125a	UCCUGAGACCCUUUAACUGUG (SEQ ID NO:151)		1	1
	miR-125b	UCCUGAGACCCU--AACUUGUGA (SEQ ID NO:152)		1	
	miR-126	UCGUACCGUGAGUAAUAUGC (SEQ ID NO:153)	4	1	
	miR-127	UCGGAUCCGUCUGAGCUUGGCU (SEQ ID NO:154)		1	
	miR-128	UCACAGUGAACCGUCUCUUUU (SEQ ID NO:155)		2	2 2
20	miR-129	CUUUUUUCGGUCUGGGCUUGC (SEQ ID NO:156)		1	
	miR-130	CAGUGCAAUGUUAAAAGGGC (SEQ ID NO:157)		1	
	miR-131	UAAAGCUAGAUAACCGAAAGU (SEQ ID NO:158)		1	1 1
	miR-132	UAACAGUCUACAGCCAUGGUUGU (SEQ ID NO:159)		1	

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	miR-133	UUGGUCCCCUUAACCAGCUGU (SEQ ID NO:160)	4			1	
	miR-134	UGUGACUGGUUGACCAGAGGA (SEQ ID NO:161)				1	
	miR-135	UAUGGCUUUUUAUCCUAUGUGAA (SEQ ID NO:162)				1	
	miR-136	ACUCCAUUUGUUUUGAUGAUGGA (SEQ ID NO:163)				1	
5	miR-137	UAUUGCUUAAGAAUACGCGUAG (SEQ ID NO:164)				1	1
	miR-138	AGCUGGUGUUGUGAAUC (SEQ ID NO:165)				1	
	miR-139	UCUACAGUGCACGUGUCU (SEQ ID NO:166)				1	1
	miR-140	AGUGGUUUUACCCUAUGGUAG (SEQ ID NO:167)			1		
	miR-141	AACACUGUCUGGUAAGAUGG (SEQ ID NO:168)		1	1		1
10	miR-142-s	CAUAAAGUAGAAAGCACUAC (SEQ ID NO:169)			1	1	
	miR-142-as ^b	UGUAGUGUUUCCUACUUUAUGG (SEQ ID NO:170)		1	1	6	
	miR-143	UGAGAUGAAGCACUGUAGCUCA (SEQ ID NO:171)	3	7		2	1
	miR-144	UACAGUAUAGAUGAUGUACUAG (SEQ ID NO:172)	2			1	
	miR-145	GUCCAGUUUCCCAGGAAUCCCUU (SEQ ID NO:173)	1				
15	miR-146	UGAGAACUGAAUCCAUUGGGUUU (SEQ ID NO:174)	1				
	miR-147	GUGUGUGGAAUUCUUCUGCC (SEQ ID NO:175)		1			
	miR-148	UCAGUGCACUACAGAACUUUGU (SEQ ID NO:176)		1			
	miR-149	UCUGGCUCGUGUCUUCACUCC (SEQ ID NO:177)	1				
	miR-150	UCUCCCAACCCUUGUACCAGUGU (SEQ ID NO:178)				1	
20	miR-151	CUAGACUGAGGCUCUUGAGGU (SEQ ID NO:179)				1	
	miR-152	UCAGUGCAUGACAGAACUUGG (SEQ ID NO:180)				1	
	miR-153	UUGCAUAGUCACAAAAGUGA (SEQ ID NO:181)					1
	miR-154	UAGGUUAUCCGUGUUGCCUUG (SEQ ID NO:182)					1

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miR-155

UUAUUGCUGAAUUGUGAUAGGGG
(SEQ ID NO:183)

1

5 The originally described miR-30 was renamed to miR-30a-as in order to distinguish it from the miRNA derived from the opposite strand of the precursor encoded by the mir-30a gene. miR-30a-s is equivalent to miR-97 [46].

A 1-nt length heterogeneity is found on both 5' and 3' end. The 22-nt miR sequence is shown, but only 21-nt miRNAs were cloned.

10

Table 4

Mouse and human miRNAs. The sequences indicated represent the longest miRNA sequences identified by cloning. The 3' terminus of miRNAs is often truncated by one or two nucleotides. miRNAs that are more than 85% identical in sequence (i.e. share 18 out of 21 nucleotides) or contain 1- or 2-nucleotide internal deletions are referred to by the same gene number followed by a lowercase letter. Minor sequence variations between related miRNAs are generally found near the ends of the miRNA sequence and are thought to not compromise target RNA recognition. Minor sequence variations may also represent A to G and C to U changes; which are accommodated as G-U wobble base pairs during target recognition. Mouse brains were dissected into midbrain, mb, cortex, cx, cerebellum, cb. The tissues analyzed were lung, ln; liver, lv; spleen, sp; kidney, kd; skin, sk; testis, ts; ovary, ov; thymus, thy; eye, ey; cortex, ct; cerebellum, cb; midbrain, mb. The human osteosarcoma cells SAOS-2 cells contained an inducible p53 gene (p53-, uninduced p53; p53+, induced p53); the differences in miRNAs identified from induced and uninduced SAOS cells were not statistically significant.

[illegible]

miR-C15	UACCACAGGGUAGAACCCAGGA	1	(SEQ ID NO.198)
miR-C16	AACUGGCCUACAAAGUCCAG	1	(SEQ ID NO.199)
miR-C17	UGUACAGCAACUCCAUGUGGA	1	(SEQ ID NO.200)
miR-C18	UAGCAGCACAGAAAUUUGGC	2	(SEQ ID NO.201)
5 miR-C19	UAGGUAGUUUCAUGUUGUGG	1	(SEQ ID NO.202)
miR-C20	UUCACCACCUUCUCCACCAGC	1	(SEQ ID NO.203)
miR-C21	GGUCCAGAGGGGAGUAGG	1	(SEQ ID NO.204)
miR-C22	CCCAGUGUUCAGACUACCUGUU	1	(SEQ ID NO.205)
miR-C23	UAAUACUGCCUGGUAAUGAGAC	2	(SEQ ID NO.206)
10 miR-C24	UACUCAGUAAAGGCAUUGUUCU	1	(SEQ ID NO.207)
miR-C25	AGAGGUUAGCGGCAUGGGAAGA	1	(SEQ ID NO.208)
miR-C26	UGAAUUGUUUAGGACCACUAG	1	(SEQ ID NO.209)
miR-C27	UCCCCUUGUCAUCCUAUGCCUG	1	(SEQ ID NO.210)
miR-C28	UCCUUCAUUCCACCGGAGUCUG	1	(SEQ ID NO.211)
15 miR-C29	GUGAAUUGUUUAGGACCACUAGA	2	(SEQ ID NO.212)
miR-C30	UGGAAUGUAAAGGAAGUGUGUGG	2	(SEQ ID NO.213)
miR-C31	UACAGUAGUCUGCACAUUGGUU	1	(SEQ ID NO.214)
miR-C32	CCCUGUAGAACCGAAUUGUGU	1	(SEQ ID NO.215)
miR-C33	AACCCGUAGAUCCGAACUUGUGAA	1	(SEQ ID NO.216)
20 miR-C34	GCUUCUCCUGGCUCUCCUCCUC	1	(SEQ ID NO.217)

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Table 5

D. melanogaster miRNA sequences and genomic location. The sequences given represent the most abundant, and typically longest miRNA sequences identified by cloning. It was frequently observed that miRNAs vary in length by one or two nucleotides at their 3'-terminus. From 222 short RNAs sequenced; 69 (31%) corresponded to miRNAs, 103 (46%) to already characterized functional RNAs (rRNA, 7SL RNA, tRNAs), 30 (14%) to transposon RNA fragments, and 20 (10%) sequences with no database entry. RNA sequences with a 5'-guanosine are likely to be underrepresented due to the cloning procedure (8). miRNA homologs found in other species are indicated. Chromosomal location (chr.) and GenBank accession numbers (acc. nb.) are indicated. No ESTs matching miR-1 to miR-14 were detectable by database searching.

miRNA	sequence (5' to 3')	chr., acc. nb.	remarks
miR-1	UGGA AUGUAAAGAAGUAUGGAG (SEQ ID NO:58)	2L, AE003667	homologs: <i>C. briggsae</i> , G20U, AC87074; <i>C.elegans</i> G20U, U97405; mouse, G20U, G22U, AC020867; human, chr. 20, G20U, G22U, AL449263; ESTs: zebrafish, G20U, G22U, BF157-601; cow, G20U, G22U, BE722-224; human, G20U, G22U, AJ220268
miR-2a	UAUCACAGCCAGCTUUGAUGAGC (SEQ ID NO:59)	2L, AE003663	2 precursor variants clustered with a copy of <i>mir-2b</i>
miR-2b	UAUCACAGCCAGCTUUGAGGAGC (SEQ ID NO:60)	2L, AE003620 2L, AE003663	2 precursor variants
miR-3	UCACUGGGCAAAGUGUGUCUCA (SEQ ID NO:61)	2R, AE003795	In cluster <i>mir-3</i> to <i>mir-6</i>
miR-4	AUAAAGCTAGACAACCAUUGA (SEQ ID NO:62)	2R, AE003795	In cluster <i>mir-3</i> to <i>mir-6</i>

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	miR-5	AAAGGAACGAUCGUUGUGAUAUG (SEQ ID NO:63)	2R, AE003795	in cluster <i>mir-3</i> to <i>mir-6</i>
	miR-6	UAUCACAGUGGCUGUUCUUUUU (SEQ ID NO:64)	2R, AE003795	in cluster <i>mir-3</i> to <i>mir-6</i> with 3 variants
5	miR-7	UGGAAGACTAGUGAUUUUGUUGU (SEQ ID NO:65)	2R, AE003791	homologs: human, chr. 19 AC006537, EST BF373391; mouse chr. 17 AC026385, EST AA881786
	miR-8	UAUAUCUGUCAGGUAAGAUGUC (SEQ ID NO:66)	2R, AE003805	
10	miR-9	UCUUUGGUUAUCUAGCUGUAUGA (SEQ ID NO:67)	3L, AE003516	homologs: mouse, chr. 19, AF155142; human, chr. 5, AC026701, chr. 15, AC005316
	miR-10	ACCCUGUAGAUCCGAAUUUGU (SEQ ID NO:68)	AE001574	homologs: mouse, chr 11, AC011194; human, chr. 17, AF287967
	miR-11	CAUCACAGUCUGAGUUCUUGC (SEQ ID NO:69)	3R, AE003735	intronic location
15	miR-12	UGAGUAUUACAUCAGGUACUGGU (SEQ ID NO:70)	X, AE003499	Intronic location
	miR-13a	UAUCACAGCCAUUUUGACGAGU (SEQ ID NO:71)	3R, AE003708 X, AE003446	<i>mir-13a</i> clustered with <i>mir-13b</i> on chr. 3R
20	miR-13b	UAUCACAGCCAUUUUGAUGAGU (SEQ ID NO:72)	3R, AE003708	<i>mir-13a</i> clustered with <i>mir-13b</i> on chr. 3R
	miR-14	UCAGUCUUUUUCUCUCUCCUA (SEQ ID NO:73)	2R, AE003833	no signal by Northern analysis

Table 6

Human miRNA sequences and genomic location. From 220 short RNAs sequenced, 100 (45%) corresponded to miRNAs, 53 (24%) to already characterized functional RNAs (rRNA, snRNAs, tRNAs), and 67 (30%)

5 sequences with no database entry. For legend, see Table 1.

miRNA	sequence (5' to 3')	chr. or EST, acc. nb.	remarks*
10 let-7a	UGAGGUAGUAGGUUGUAUAGUU (SEQ ID NO:75)	9, AC007924, 11, AP001359, 17, AC087784, 22, AL049853	sequences of chr 9 and 17 identical and clustered with <i>let-7f</i> , homologs: <i>C. elegans</i> , AF274345; <i>C. briggsae</i> , AF210771, <i>D. melanogaster</i> , AE003659
let-7b	UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:76)	22, AL049853†, ESTs, AI382133, AW028822	homologs: mouse, EST AI481799; rat, EST, BE120662
let-7c	UGAGGUAGUAGGUUGUAUGGUU (SEQ ID NO:77)	21, AP001667	Homologs: mouse, EST, AA575575
15 let-7d	AGAGGUAGUAGGUUGCAUAGU (SEQ ID NO:78)	17, AC087784, 9, AC007924	identical precursor sequences
let-7e	UGAGGUAGGAGGUUGUAUAGU (SEQ ID NO:79)	19, AC018755	
20 let-7f	UGAGGUAGUAGAUGUAUAGUU (SEQ ID NO:80)	9, AC007924, 17, AC087784, X, AL592046	sequences of chr 9 and 17 identical and clustered with <i>let-7a</i>
miR-15	UAGCAGCACATAAUGGUUUGUG (SEQ ID NO:81)	13, AC069475	in cluster with <i>mir-16</i> homolog
miR-16	UAGCAGCACGTAATAUUGGCG (SEQ ID NO:82)	13, AC069475	in cluster with <i>mir-15</i> homolog

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	miR-17	ACUGCAGUGAAGGCACUUGU (SEQ ID NO:83)	13, AL138714	in cluster with <i>mir-17</i> to <i>mir-20</i>
	miR-18	UAAGGUGCAUCUAGUGCAGADA (SEQ ID NO:84)	13, AL138714	in cluster with <i>mir-17</i> to <i>mir-20</i>
5	miR-19a	UGUGCAAUUCUAUGCAAACUG A (SEQ ID NO:85)	13, AL138714	in cluster with <i>mir-17</i> to <i>mir-20</i>
	miR-19b	UGUGCAAUCCAUUGCAAACUG A (SEQ ID NO:86)	13, AL138714, X, AC002407	in cluster with <i>mir-17</i> to <i>mir-20</i>
	miR-20	UAAAGUGCUUAGUGCAGGUA (SEQ ID NO:87)	13, AL138714	in cluster with <i>mir-17</i> to <i>mir-20</i>
10	miR-21	UAGCUUAUCAGACUGAUGUUGA (SEQ ID NO:88)	17, AC004686, EST, BF326048	homologs: mouse, EST, AA209594
	miR-22	AAGCUGCCAGUUGAAGAACUGU (SEQ ID NO:89)	ESTs, AW961681†, AA456477, AI752503, BF030303, HS1242049	human ESTs highly similar; homologs: mouse, ESTs, e.g. AA823029; rat, ESTs, e.g. BF543690
15	miR-23	AUCACAUUGCCAGGGAUUGCC (SEQ ID NO:90)	19, AC020916	homologs: mouse, EST, AW124037; rat, EST, BF402515
	miR-24	UGGCUCAGUUCAGCAGGAACAG (SEQ ID NO:91)	9, AF043896, 19, AC020916	homologs: mouse, ESTs, AA111466, AI286629; pig, EST, BE030976
20	miR-25	CAUUGCACUUGUCUGGUCUGA (SEQ ID NO:92)	7, AC073842, EST, BE077684	human chr 7 and EST identical; highly similar precursors in mouse ESTs (e.g. AI595464); fish precursor different STS: G46757
	miR-26a	UUCAAGUAAUCCAGGAUAGGCU (SEQ ID NO:93)	3, AP000497	

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	miR-26b	UUCAAGUAAUUCAGGAUAGGUU (SEQ ID NO:94)	2, AC021016	
	miR-27	UUCACAGUGGCUAAGUUCGCU (SEQ ID NO:95)	19, AC20916	U22C mutation in human genomic sequence
5	miR-28	AAGGAGCUCACAGUCUAUUGAG (SEQ ID NO:96)	3, AC063932	
	miR-29	CUAGCACCAUCUGAAAUCGGUU (SEQ ID NO:97)	7, AF017104	
	miR-30	CUUUCAGUCGGAUGUUUGCAGC (SEQ ID NO:98)	6, AL035467	
10	miR-31	GGCAAGAUGCUGGCAUAGCUG (SEQ ID NO:99)	9, AL353732	
	miR-32	UAUUGCACAUUACUAAGUUGC (SEQ ID NO:100)	9, AL354797	not detected by Northern blotting
15	miR-33	GUGCAUUGUAGUUGCAUUG (SEQ ID NO:101)	22, Z99716	not detected by Northern blotting

*If several ESTs were retrieved for one organism in the database, only those with different precursor sequences are listed.

20 tprecursor structure shown in Fig. 4.

Claims

1. Isolated nucleic acid molecule comprising

5 (a) a nucleotide sequence as shown in Table 1, Table 2, Table 3 or Table 4 or a precursor thereof as shown in Figure 3, Figure 4 or Figure 7.

10 (b) a nucleotide sequence which is the complement of (a),

(c) a nucleotide sequence which has an identity of at least 80% to a sequence of (a) or (b) and/or

15 (d) a nucleotide sequence which hybridizes under stringent conditions to a sequence of (a), (b) and/or (c).

2. The nucleic acid molecule of claim 1, wherein the identity of sequence (c) is at least 90%.

20 3. The nucleic acid molecule of claim 1, wherein the identity of sequence (c) is at least 95%.

25 4. The nucleic acid molecule of any one of claims 1-3, which is selected from miR 1-14 as shown in Table 1 or miR 15-33 as shown in Table 2 or miR 1-155 as shown in Table 3 or miR-C1-34 as shown in Table 4 or a complement thereof.

30 5. The nucleic acid molecule of any one of claims 1-3, which is selected from mir 1-14 as shown in Figure 3 or let 7a-7f or mir 15-33, as shown in Figure 4 or let 7a-i or mir 1-155 or mir-c1-34, as shown in Figure 7 or a complement thereof.

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6. The nucleic acid molecule of any one of claims 1-4 which is a miRNA molecule or an analog thereof having a length of from 18-25 nucleotides.

7. The nucleic acid molecule of any one of claims 1-3 or 5, which is a miRNA precursor molecule having a length of 60-80 nucleotides or a DNA molecule coding therefor.

8. The nucleic acid molecule of any one of claims 1-7, which is single-stranded.

9. The nucleic acid molecule of any one of claims 1-7, which is at least partially double-stranded.

10. The nucleic acid molecule of any one of claims 1-9, which is selected from RNA, DNA or nucleic acid analog molecules.

11. The nucleic acid molecule of claim 10, which is a molecule containing at least one modified nucleotide analog.

12. The nucleic molecule of claim 10 which is a recombinant expression vector.

13. A pharmaceutical composition containing as an active agent at least one nucleic acid molecule of any one of claims 1-12 and optionally a pharmaceutically acceptable carrier.

14. The composition of claim 13 for diagnostic applications.

15. The composition of claim 13 for therapeutic applications.

16. The composition of any one of claims 13-15 as a marker or a modulator for developmental or pathogenic processes.

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17. The composition of claim 13 as a marker or modulator of developmental disorders, particularly cancer, such a B-cell chronic leukemia.

18. The composition of any one of claims 13-15 as a marker or modulator of gene expression.

19. The composition of claim 18 as a marker or modulator of the expression of a gene, which is at least partially complementary to said nucleic acid molecule.

20. A method of identifying microRNA molecules or precursor molecules thereof comprising ligating 5'- and 3'-adapter molecules to the ends of a size-fractionated RNA population, reverse transcribing said adapter-containing RNA population and characterizing the reverse transcription products.

Fig. 1 A

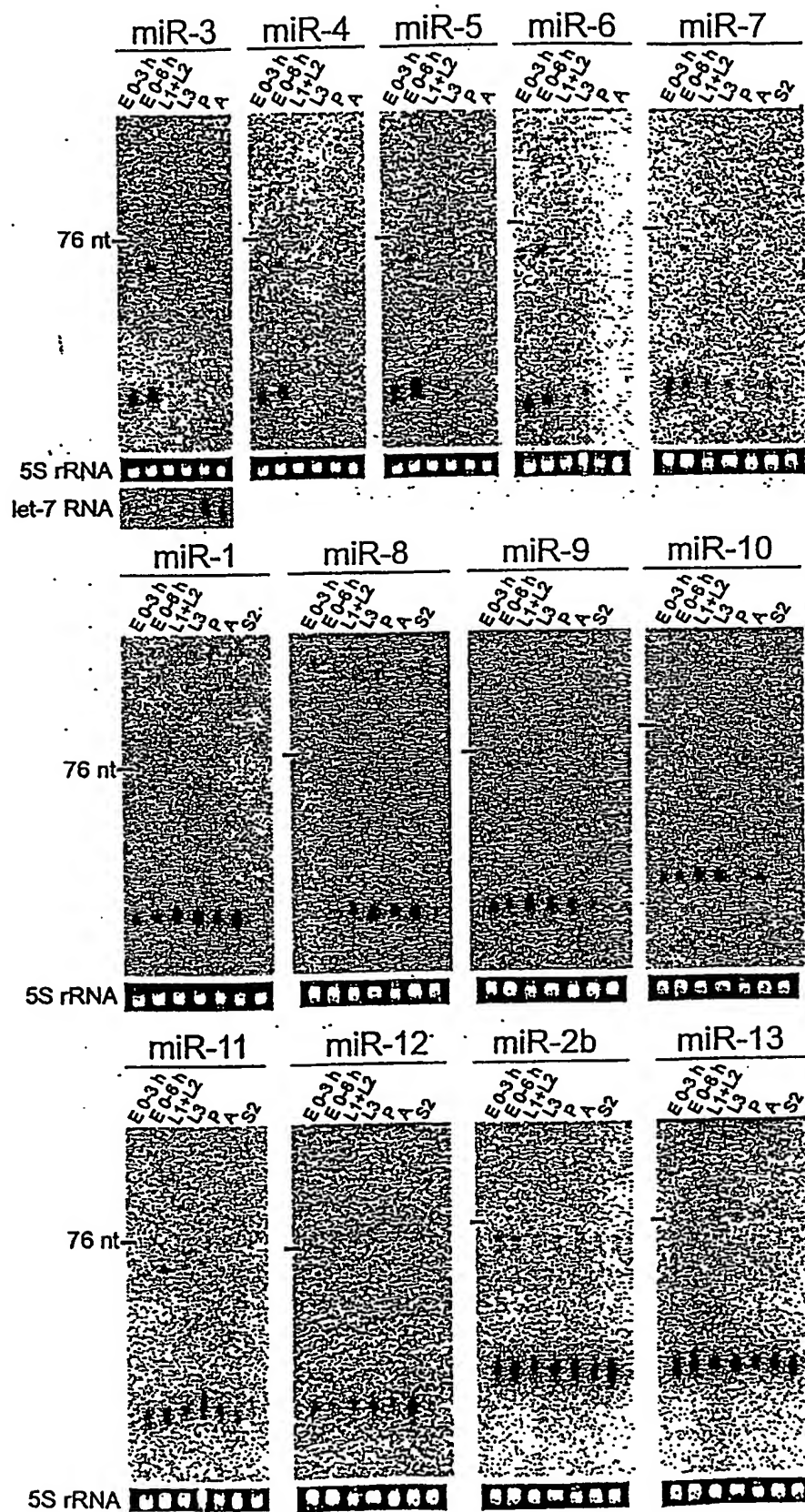


Fig. 1 B

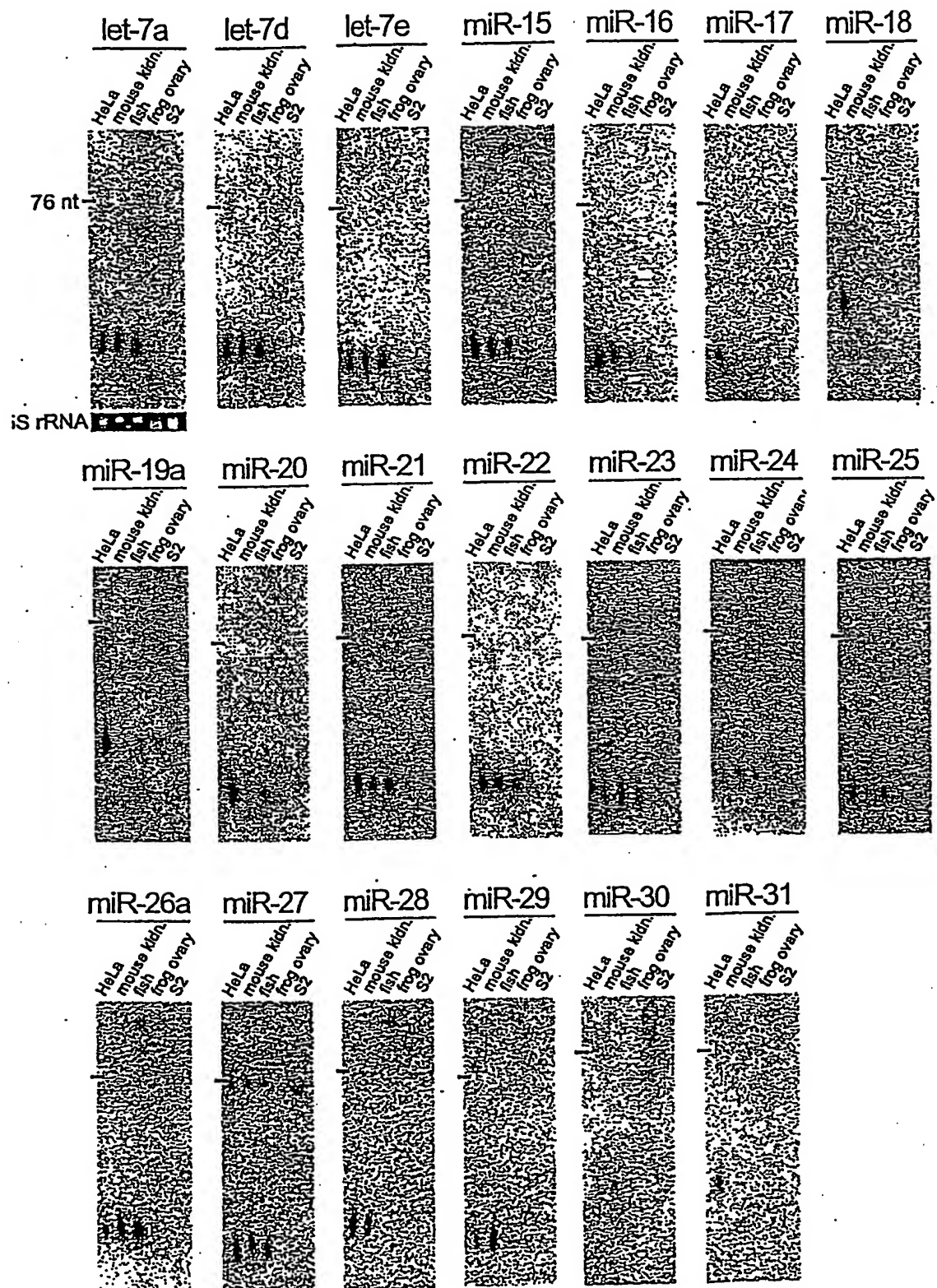


Fig. 2

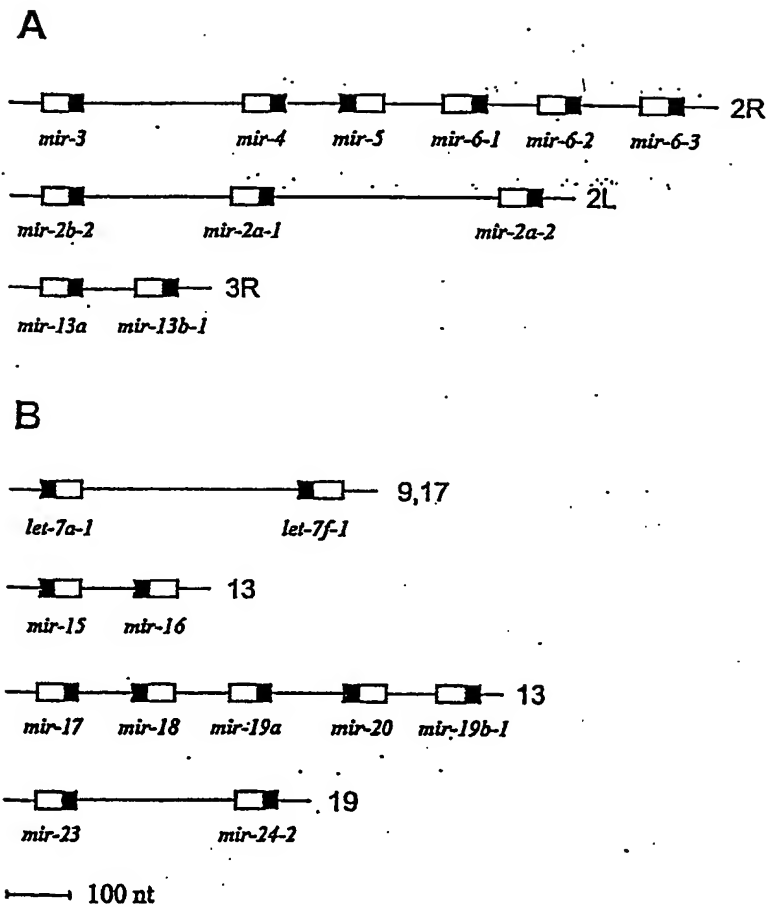


Fig. 4

let-7a-1
chr. 9,17

let-7a-2
chr. 11

let-7a-3
chr. 22

let-7b

let-7c

let-7d

let-7e

let-7f-1
chr. 9,17

let-7f-2
chr. X

mir-15

mir-16

mir-17

mir-18

mir-19a

mir-19b-1
chr. 13

mir-19b-2
chr. X

mir-20

mir-21

mir-22

mir-23

mir-24-1
chr. 9

mir-24-2
: chr. 19

mir-25

mir-26a

mir-26b

mir-27

mir-28

mir-29

mir-30

mir-31

mir-32

mir-33

Fig. 5

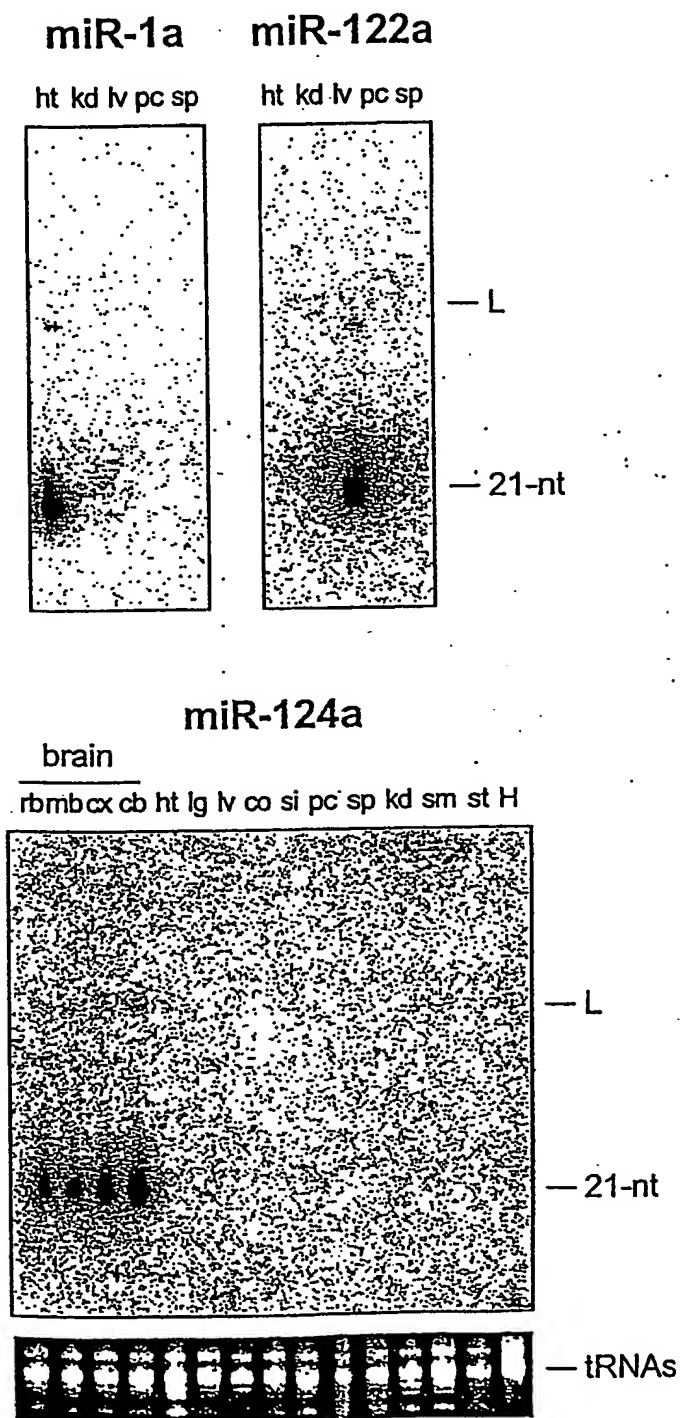


Fig. 5 (cont.)

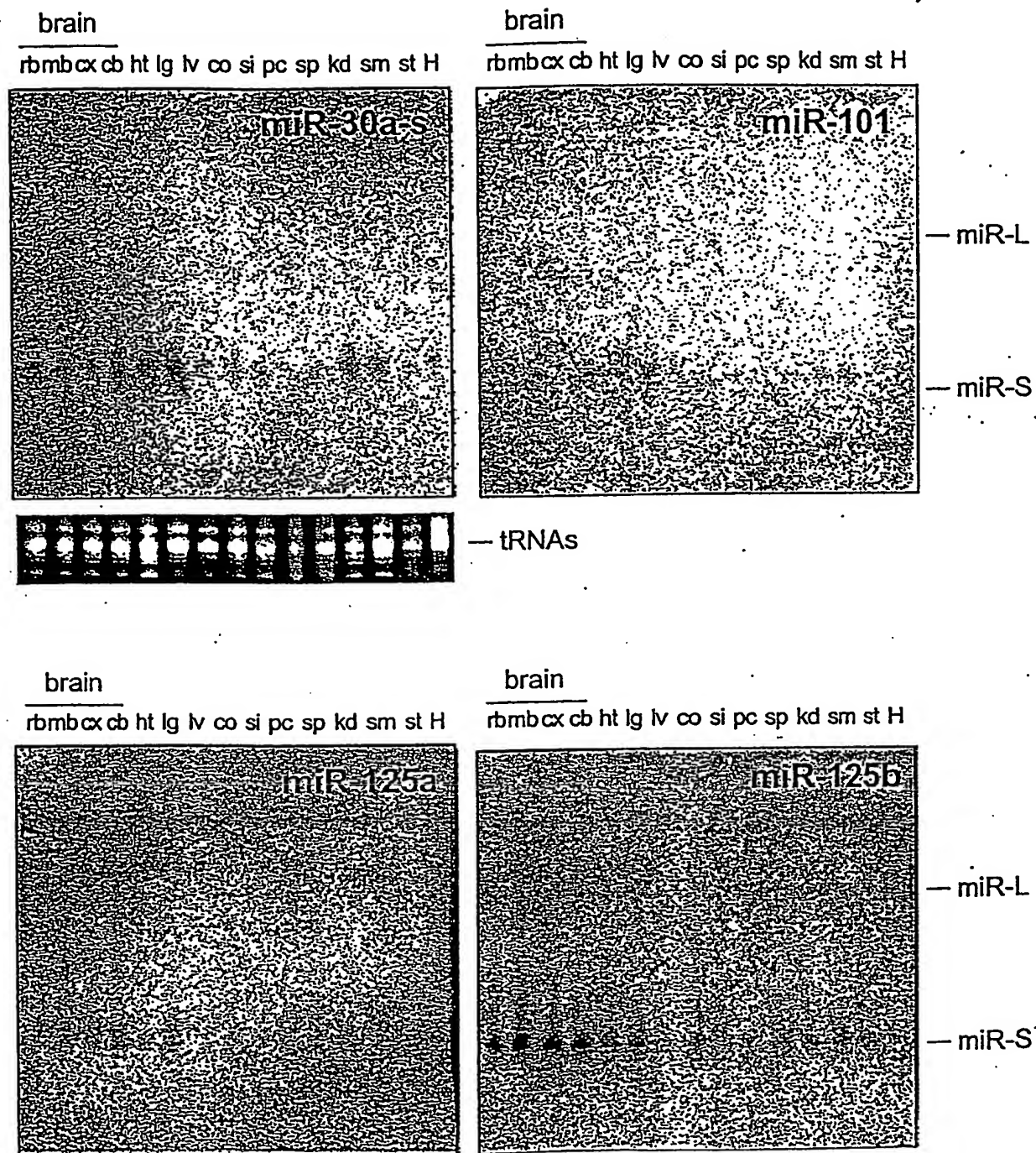


Fig. 5 (cont.)

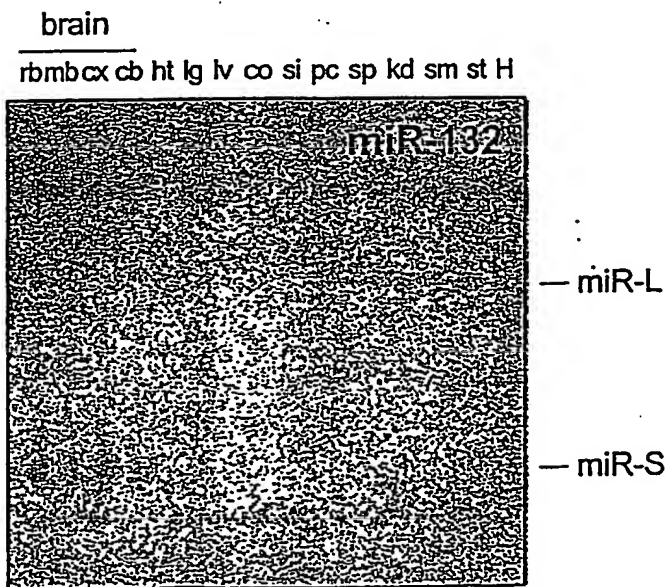
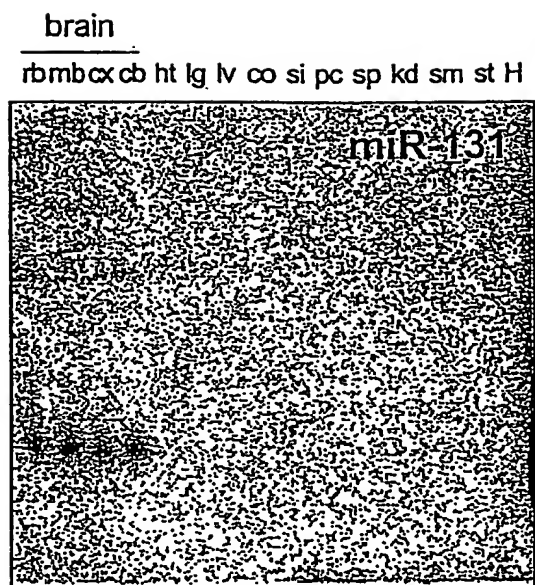
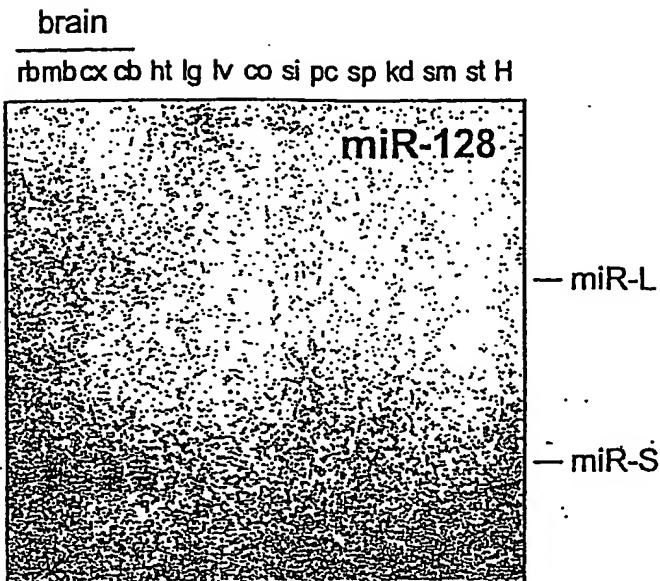
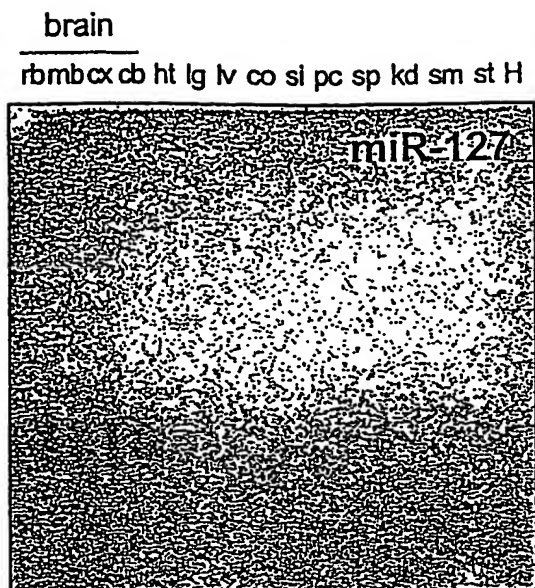
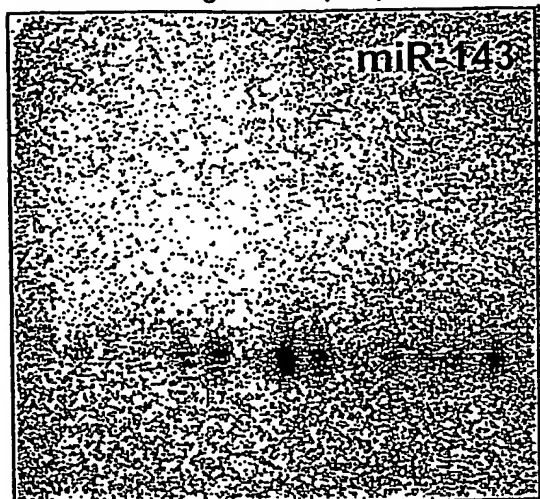


Fig. 5 (cont.)

brain

rb mbcx cb ht lg lv co si pc sp kd sm st H



— miR-L

— miR-S

Fig. 6

A

C. elegans lin-4*D. melanogaster* miR-125*M. musculus*/*H. sapiens* miR-125b*M. musculus*/*H. sapiens* miR-125a

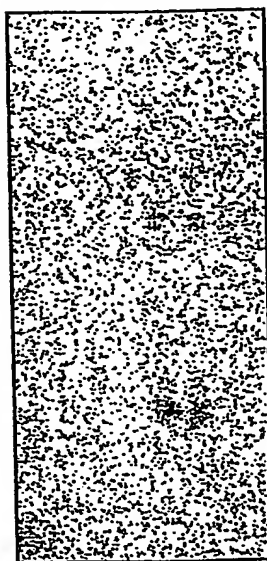
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UCCCUAGAGACCCU--AACUUGUGA

UCCCUAGAGACCCU--AACUUGUGA

UCCCUAGAGACCCUUAACCUGUGA

B

036
L1+L2
L3R7S2

— L

— 21-nt

Fig. 7

name	sequence	structure
let-7a-1	UGAGGUAGUAGGUUGUAUAGUU	UG U UGAG ACA C CAC UGGGA GAGUAGUAGGUUAUAGUU GUC CCCA C GUG AUCCU UUCUGUCAUCAUAUACAA UAG GGU A CA - - - - - A - - - C
let-7a-2	UGAGGUAGUAGGUUGUAUAGUU	UU G U UAGAUAUAC AA AGG GAG UAG AGGUUGUAUAGUU AUC G UCC UUC AUC UCCGACAUGUCA A UAG G U- G C - - - - - AG
let-7a-3	UGAGGUAGUAGGUUGUAUAGUU	U GAGUAGUAGGUUGUAUAGUU U GGG GAGUAGUAGGUUGUAUAGUU UGGG C UCC UUCUGUCAUCAUAUACAA GUCCCG C U UAGGUUAUC U
let-7b	UGAGGUAGUAGGUUGUGUGGUU	GG U - - A - - - - UG CCGGG GAGUAGUAGGUUGUGUGGUU UC GGCAG GUCCC UUCGUCAUCCACAUUAUCAA AG CCGUU A - - - - - U AAGGCUC GU
let-7c	UGAGGUAGUAGGUUGUAUAGUU	A UU G U UA G UA AC GC UCCGG GAG UAG AGGUUGUAUAGUU GA U C CG AGGUUC UUC AUC UCCACAUGUCA UU A G C - CU G U - - G GG UC
let-7d	AGAGGUAGUAGGUUGCAUAGU	A C UUA - - - - GG CCUAGGA GAGUAGUAGGUUG AUAGUU GGCAG GGAUUCU UUCGUGGCCAGC UAUA A CCGUU A - - - - - UUGAGGAACA UU
let-7e	UGAGGUAGGAGGUUGUAUAGU	C CU G U GGA - - - A CC GGG GAG UAGGAGGUUGUAUAGU GA GG C GG CCC UUC AUCCUCCGCAUAUCA CU CC A A CU G - - AGAGGA A C

Fig. 7 (cont.)

let-7f-1	UGAGGUAGUAGAUUGUUAUGUU	AGU GAGGUAGUAGAUUGUUAUGUU UG UCAG GAGGUAGUAGAUUGUUAUGUU GGGUAG AGUC UUCCGUUAUCUAACAUAUCAUA UCCCAUU A CC- GAGGACUUG UUU
let-7f-2	UGAGGUAGUAGAUUGUUAUGUU	U UCAU CUGUGGGA GAGGUAGUAGAUUGUUAUGUU A GGCACCCU UUCUGUCAUCUGACAUAUCAU C UAGA ACCC
let-7g	UGAGGUAGUAGUUUGUACAGUA	A U UGAGG A- A A CC GGC GAGGUAGU GGUUGUACAGUU GUCU UG UACC C GG CCG UUCCGUCA CGGACAUGUCA UAGA AC AUGG C A - C - - - - - GG - C
let-7h	UGAGGUAGUAGUGUGUACAGUU	
let-7i	UGAGGUAGUAGUUUGUGCU	U U UGUG CUGGC GAGGUAGUAGUUUGUGCU GG CCGU GAUCG UUCCGUCAUCGAACGCG CAA UC GCCCG A U UAGAGGUG - UUAC
miR-1	UGGAUUGUAAAGAGUAUGGAG	A UUUGAGA C A - AUA UUC GCC GUUCCAUGCUUC UUGCAUUC AUA GUU GAG CGG CGAGGUAGAAG AAUGUAAG UAU CGA U - UCUAAG A G A ACU
miR-1b	UGGAUUGUAAAGAGUAUGUAA	A GC - - - - AC UGGGA ACAUACUUCUUUAUAU CCAUA UGG ACUCU UGUAGUAGAGAAUGUA GGUAU AUC C A A- CGA GU AL449263.5

Fig. 7 (cont.)

miR-1c	UGGAUGUAAAGAGUAUGUAC	
miR-1d	UGGAUGUAAAGAGUAUGUAUU	<p> C GC UGAACC U GCUUGGGA ACAURCUUCUUUAUU CCAUA. U CGGACUUU UGUAGAAGAAUGUA GUUAU G A A- CGAAUC </p>
miR-2a-1	UAUCACAGCCAGCUUUGAUGAGC	<p> - - - A AUUUC UU GCUGGGCUC UCAAG UGGUUGUGA AUGC CGC \ CGAUUGGAG AGUUUC ACCGACACU UACG . GCG U U g A ----- CG </p>
miR-2a-2	UAUCACAGCCAGCUUUGAUGAGC	<p> A C -- -- GAUAC AUCU AGC UCAUCAAG UGGUUGUGUAUUG \ UAGG UCG AGUAGUUU ACCGACACUAUAC C A - - CG GCAC </p>
miR-2b-1	UAUCACAGCCAGCUUUGAGGAGC	<p> U UG - - A C----- U CU CAAC UCUUCAAG UGGC GUGA . AUGUUG C GG GUUG AGGAGUUUC ACCG CACU UUAUAC A C CG G A AUACU A </p>
miR-2b-2	UAUCACAGCCAGCUUUGAGGAGC	<p> A - - A UUU--- CUU UUGUGUC UUCUCAAAG UGGUUGUGA AUG GC U AGCGCAG GAGGAGUUUC ACCGACACU UAC CG U C G A UUAUC UAU </p>
miR-3	UCACUGGGCAAGUGUGUCUCA	<p> C C G U UUCA GAUC UGGGAUGCAU UUGU CAGU AUGU \ CUAG ACUCUGUGUG AACG GUCA UACA A A A G C CUCU </p>

Fig. 7 (cont.)

miR-9	UCUUUGGUUAUCUAGCUGUAGA	<p> - U U UAU G - GAU GCUA UGUUG CUUUGU CUAGCU UAUGA GU A CGAU AUAU GAAGCA GAUGA AUACU CA A U U UUC A G AUA </p>
miR-10	ACCCUGUAGAUCCGAAUUUGU	<p> CU - G U AUACU CCACGU ACC CU UAGA CCGAAUUUGUUU A GGUGUG UGG GA AUEU GGCUUAACACGGA G UU A G U AUUUC </p>
miR-11	CAUCACAGUCUGAGUUCUUGC	<p> U UCU CCC U ACU GCACUUG CAAGAACUU CUGUGA GCG GU U CGUGAGU GUUCUUGAG GACACU CGO CG A C UCU A-- - AAA </p>
miR-12	UGAGUAUUACAUCAGGUACUGGU	<p> UG U C - GCCUU UACGGU AGUAAU ACAU AGGUACUUGU GU A GUGCCG UCAUA UGUA UUCAUGACCA CA A CA C - A ACCUA </p>
miR-13a	UAUCACAGCCAUUUUGAUGAGU	<p> U C - A UC-- CU UACG AACUC UCAAAG GGUUGUGA AUG GA A GUGC UUGAG AGUUUU CCGACACU UAC CU U U U A A UCAU AU </p>
miR-13b-1	UAUCACAGCCAUUUUGACGAGU	<p> UG- U ACU UAUU CCA UCGUUAUUUG UUGUGA UAUG C GGU AGCAGUUUUAC GACACU AUAC A UUG C ---- UAAC </p>
miR-13b-2	UAUCACAGCCAUUUUGACGAGU	<p> UAUU G A GCUA UU AAC CGUCAAUUG CUGUGA UGUGGA U UUG GCAGUUUUAC GACACU AUACUU G GU-- A C ---- CA </p>

Fig. 7 (cont.)

miR-14	UCAGUCUUUUUCUCUCUCCUA	UGUGGGAG GAGA GGGGACU ACUGU \ A AUAUCCUC CUCU UUUUCUGA UGAUA A U U C AAUU
miR-15a	UAGCAGCACAUAAUGGUUUUGUG	GAGUAAAAGUA UA GA U CCUUG GCAGCACA AUGGUUUUGU UUU \ GGAAC CGUCUGU UACCGGACGU AAA G AUAAAAACUC UA GG A
miR-15b	UAGCAGCACAUCAUGGUUUUACA	U C C C A A ACA CUG AGCAGCA AU AUGGUUU CAU CU \ GAU UCGUCGU UA UACUAAG GUA GA G C U U C - ACU
miR-16	UAGCAGCACGUAAAUAUUGGCG	AG C - A CGUUA UCUA GUCAGC UGC UUAGCAGCAC GU AAUAUUGG AGAU \ CAGUUG AUG AGUCGUCGUG CA UUAUGACC UCUA A GA A U A ----- UUA
miR-16	only different precursor	UC CU UA C AG AAU GU CACU AGCAGCAGC AAUAUUGG GU UGA A CA GUGA UCGUGUGU UUAUAACC CA AUU U GU UU CA A A... AUA
miR-17	ACUGCAGUGAAGGCACUUGU	GA CA- A G G - AUA GUCA AUAUGU AAGUGCUU CA UGCAG UAG UG \ CAGU UAUUACG UUCACGGA GU AGCUC AUC AC U GG AUG A G - U GUG
miR-18	UAAGGUGCAUCUAGUGCAGUA	CU U C U A UGAR AG UGUU AAGG GCAU UAG GCAG UAG GU A ACGG UUCC CGUG AUC CGUC AUC CG U UC U A C - UA-- AU

Fig. 7 (cont.)

miR-19a	UGUGCAAUUCUAUGCAAACUGA	<p>U U --- AGA GCAG CC CUGUAGUUUUGCAUAG UUGCAC UACA \</p> <p>C U UA UUG AAG CGUC GG GGUAGUCAAAACGUAC AACGUG AUGU A</p>
miR-19b-1	UGUGCAAUUCUAUGCAAACUGA	<p>UU UC UGUGUG CACUG CUUUGGUUAGUUUGCA GG UUUGCA CAGC \</p> <p>--- A U UCUUUAU GUGAU GGUGUCAGUCAAAACGU CC AAACGU GUCC A</p>
miR-19b-2	UGUGCAAUUCUAUGCAAACUGA	<p>CUAC UUCA U ACAUG UUACAAUUAUUGUUGCA GG UUUGCAU GCGUAUA A</p> <p>----- A U UCGG G UGUAU AGUGUAGUCAAAACGU CC AAACGU UGUUAU U</p>
miR-20	UAAAGUGCUUAUAGUGCAGGUAG	<p>C A- G - - UU GUAG ACU AAGUGCUUAUAGUGCAG UAG UG U</p> <p>A AA - - U UG CGUC UGA UUCACGAGUAUACGUC AUC AU A</p>
miR-21	UAGCUUAUCAGACUGAUGUUGA	<p>A A A A U AA UGUCGGGUAAGCUUAUC GACUG UGUUG CUGU G \</p> <p>- C - - UC ACAGUCUGUGGGUAG CUGAC ACAAC GGUA C U</p>
miR-22	AAGCUGCCAGUUGAAGAACUGU	<p>U CC - A U CCUG GGC GAG GCAGUAGUUUCUUCAG UGGCA GCUUUA GU \</p> <p>U C- G - - ACCC CCG CUC CGUUGUCAAGAGUUU ACCGU CGAAU CG A</p>
miR-23a	AUCACAUUGCCAGGGAUUUC	<p>C C G G CUUC GG CGG UGGGG UUCCUGG GAUG GAUUUG C</p> <p>A A U G A ACUG CC GCC ACCUU AGGGACC UUAC CUAAAC U</p>

Fig. 7 (cont.)

miR-23b	AUCACAUUGCCAGGGAUUAACCAC	<p>C U -- C GUGACU</p> <p>GG UGC UGG GUUCCUGGCA UG UGAUUU U</p> <p>CC ACG ACC UAGGGACCGU AC ACUAAA G</p> <p>A C AU U - AUUAGA</p>
miR-24-1	UGGCUCAGUUCAGCAGGAACAG	<p>G G A UA UCUCAU</p> <p>CUCC GU CCU CUGAGCUGA UCAGU \</p> <p>GAGG CA GGA GACUUGACU GGUCA U</p> <p>A A C C- CACAUI</p>
miR-24-2	UGGCUCAGUUCAGCAGGAACAG	<p>CC CG CU- AA-- UU</p> <p>CUCUG UCC UGC ACUGAGCUG ACACAG \</p> <p>GGGAC AGG ACG UGACUCGGU UGUGUU G</p> <p>A- -- ACU CACA UG</p>
miR-25	CAUUGCACUUGUCUCGGUCUGA	<p>A AG G UU G UG ACG</p> <p>GGCC GUGUUG AGGC GAGAC G GCAAU CUGG C</p> <p>CCGG CGUGAC UCUG CUCUG C CGUUA GGUC U</p> <p>C AG G UU A CG CCG</p>
miR-26a	UUCAAGUAAUCCAGGAUAGGCU	<p>- G U U GCAG</p> <p>AGCC GUG CCUCGU CAAGUAA CCAGGAUAGGCUU G</p> <p>UCCGG CGC GGGGCA GUUCAUU GGUUCAUCCGGUA U</p> <p>G A C - ACCC</p>
miR-26b	UUCAAGUAAUUCAGGAUAGGUU	<p>GA - U UC</p> <p>CCGG CCC AGU CAAGUAAU AGGAUAGGUUG \</p> <p>GGCC GGG UCG GUUCAUUA UCUUGUCCGAC C</p> <p>AG C - CC CUGU</p>
miR-27a	UUCACAGUGGCUAAGUUCGCCU	<p>A A A U G UCCAC</p> <p>CUG GG GC GGGCUUAGCUGCU GUGAGCA GG \</p> <p>GAC CC CG CUUGAUCGGUGA CACHUGU CU A</p> <p>C C C G GAACC</p>

Fig. 7 (cont.)

miR-27b	UUCACAGUGGCUAAGUUCUG	AUGUG UGAU U AGGUGCAGAGCUUAGCUG UGG \ UCCACGUCUUGAAUCCGU GCC U GA-- UC-- U
miR-28	AAGGAGCUCACAGUCUAUUGAG	C A U ----- CC GGU CUUGCCCUCC AGGAGCUCACAGUCUA UG AGUUA U UCA GGACGGGAG UCCUCGAGUGUAGAU AC UCAGU U C G C CCUU CU
miR-29a	CUAGCACCAUCUGAARAUCCGGUU	UUU C UCRAU AUGACUGAUUUC UGGUGUU AGAG \ UAUUGGCUAAAG ACCACGA UCUU A UCU - UUAUU
miR-29b	UAGCACCAUUUGAARAUCCAGUGUU	A U GU UUAUU AGGA GCUGGUUUA AUGGUG UUAAGU \ UCUU UGACUAAAGU UACCAG GAUCUG A G U -- UUAGUG
miR-29c	UAGCACCAUUUGAARAUCCGGUUA	
miR-30a-s	UGUAAACAUCUCCUGGACUGGAAGC	A UC ----- A GCG CUGUAAACAUCUCC GACUGGAAGCU GUG A CGU GACGUUUGUAGG CUGACUUUCCGG CAC G C -- GUAAA C
miR-30a-as	CUUUCAGUCGGGAUGUUUUGCAGC	A UC ----- A GCG CUGUAAACAUCUCC GACUGGAAGCU GUG A CGU GACGUUUGUAGG CUGACUUUCCGG CAC G C -- GUAAA C

Fig. 7 (cont.)

miR-30b	UGUAAACAUCUACACUCAGC	<p>U - UCUA</p> <p>AUGUAAACAUCU ACA CUCAGCUG C</p> <p>UGCAUUUGUAGG UGU GGGUCGGU A</p> <p>- A UGCGU</p>
miR-30c	UGUAAACAUCUACACUCUCAGC	<p>UACU U ACA GUGGAA</p> <p>AGA GUAAACA CCU CUCUCAGCU A</p> <p>UCU CAUUUGU GGA GAGGGUCGA G</p> <p>UUCU C A-- AAGAAU human</p>
miR-30d	UGUAAACAUCUCCCGACUGGAAG	<p>U U CCC GUAGA</p> <p>GU GU GUAAACAUC GACUGGAAGCU C</p> <p>CA CG CGUUUGUAG CUGACUUUCGA A</p> <p>U U A-- AUCGAC chr8 human</p>
miR-31	GGCAAGAUUCUGGCAUAGCUG	<p>GA G C U- GAA</p> <p>GGAGAG GGCAA AUG UGGCAUAGC GUU C</p> <p>CCUUUC CCGUU UAC ACCGUUACG CAA U</p> <p>UA A A UC GGG</p>
miR-32	UAUUGCACAUUACUAAAUUGC	<p>U - UU C</p> <p>GGAGAUUUGCACAU ACUAAAUUGCAU G GU A</p> <p>CUUUUAUAGUGUGUG UGAUUUAACGUA C CG C</p> <p>- A UC G</p>
miR-33	GUGCAUUGUAGUUGCAUUG	<p>A UU UUCU UG</p> <p>CUGUGUGCAUUG G GC AUUGCAUG GG</p> <p>GACACUACGUGACA C UGUAAACGUAG CC G</p> <p>C UU ---- AU</p>
miR-99a	ACCGUAGAUCUGAUCUUGU	<p>A UC U G AAG</p> <p>CAUA ACCCGUAGA CGA CUUUGUG UG U</p> <p>GUGU UGGGUUUCU GCU GAACGC GC G</p> <p>C UU C - CAG</p>

Fig. 7 (cont.)

miR-99b	CACCCGUAGAACCGACCUUGCG	CC AC C ----- C GGCAC ACCGUAGA CGA CU UGCGG GG \ CUGUG UGGUGUCU GCU GA ACGCC CU C CC GU C ACAC G U
miR-101	UACAGUACUGUGAUAAACUGA	A GUCCA UCAGUUUACACAGUGUCUG UGCU U AGUCAUAGUGUCAUGAC AUGG U - AAUC
miR-122a	UGGAGUGUGACAAUGGUGUUUGU	GG C UGUCC AGCUGU AGUGUGA AAUGGUGUUUG A UCGAUA UCACACU UUACCGCAAC A AA A UAUCA woodchuck
miR-122b	UGGAGUGUGACAAUGGUGUUUGA	
miR-122a,b	UGGAGUGUGACAAUGGUGUUUG	
miR-123	CAUUAUUACUUUUGGUACGCG	A A U CGCUG C UGAC GC CAUUAUUACUU UGGUACG UGA A ACUG CG GUAAUAAUGAG GCCAUGC ACU C G C U UCAG- U
miR-124a*	UUAAGGCACGCGGUGAAUGCCA	- C A GA UAAUG CUCU G GUGUUCAC GCG CCUUGAUU U GAGA C CGUAGUG CGC GGAUUAA C A - G AC CAUUAU

Fig. 7 (cont.)

miR-124b	UUAAGGCACGCGGGGUGAAUUC	<p>CC A GA UAAUG CUCU GUGUUCAC GCG CCUUGAUU GAGA CGUAAGUG CGC GGAAUUA U AC G AC CAUAC</p> <p>AC021518</p>
miR-125a	UCCUGAGACCCUUUAACCUGUG potential lin-4 ortholog	<p>C C UA ---- A CUGGU CCUGAGA CCUU ACCUGUGA GG C GGUCCG GGUUCU GGAG UGGACACU CC G A U -- GGGA U</p>
miR-125b	UCCUGAGACCCUAACUUGUGA potential lin-4 ortholog	<p>UC C A GG- U GCCUAG CCUGAGA CCU ACUUGUGA UAU U CGGAUC GGUUCU GGA UGAACACU AUG U CA U C ACA A</p>
miR-126	UCGUACCGUGAGUAUAUAGC	<p>A U CGCUG C GC CAUUAUUAUU UGGUACG UGA A CG GUAAUAUAG GCAUAG ACU C C U UCAA- U</p>
miR-127	UCGGAUCCGUCUGAGCUUGGCU	<p>A U G G C -- AG CC GCC GCU AAGCUCAGA GG UCUGAU UC GG UGG CGG UUCGAGUCU CC AGGCUA AG A C U - G U CU AA</p>
miR-128	UCACAGUGAACCGGUCUCUUUU	<p>UUC UAG CU U GUUGGA GGGGCCG CACUGU GAGAGGU U CGACUU CUCUGGC GUGACA CUCUUUA A UUU CAA -- C</p>
miR-129	CUUUUUUCGGUCUGGGGCUUGC	<p>- C CU G UCCU C GGAU CUUUUUU GGU GGGCUU CUG CU A UCUA GAAAAAC CCA CCGGA GAC GA A U C UU G UGAU- C human</p>

Fig. 7 (cont.)

miR-130	CAGUGCAUUGUUAAAAGGCG	<p>GA GCUCUUU ACAUUGUCU CU A GUCUAAC \</p> <p>CU CGGAAAA UGUACGUGA GA G</p> <p>A U C GCCAUGU</p>
miR-131	UAAAGCUAGAUAAACCGAAAGU	<p>G C G U A</p> <p>GUU UUAU UUUGUUUACUAGCU UAUGAG GU U</p> <p>CAA AAUG AAGCCAAUAGAUGGA AUACUU UG U</p> <p>A A A C G</p>
miR-132	UAAACAGUCUACAGCCCAUGGUCGU	<p>A UUC G- G</p> <p>GGGC ACCGUGGCU GAUUGUUACU UGG \</p> <p>CCCC UGGUACCGA CUGACAAGG GCC.A</p> <p>C CAU AG A</p>
miR-133	UUGGUCUCCCUUCAACCAGCUGU	<p>A AA U A GCCUC</p> <p>GCUA AGCUGGU AA GG ACCAAUUC U</p> <p>CGAU UCGACCA UU CG UGGUUUAG U</p> <p>G AC C C GUAAC</p>
miR-134	UGUGACUGGUUGACCAGAGGGA	<p>GU U A- G GCU AC</p> <p>AGGGU GUGACUGG UG CCA AGGG GC \</p> <p>UCCCA CACUGAUC AC GGU UCCC UG U</p> <p>AC C CG G ACU- UC</p>
miR-135	UAUGGCUUUUUUUAUCCUAUGUGAA	<p>UUU UUCUUAU</p> <p>CUAUGGCUUU AUUCCUAUGUGA \</p> <p>GGUGCCGAGG UAGGGAUUAUACU U</p> <p>U- CGCUCG</p>
miR-136	ACUCCAUUUGUUUUGAUGAUGGA	<p>C UUU UUCU</p> <p>GAGGACUC AUUUG UGAUGAUGGA \</p> <p>CUUCUGAG UAAAC GCUACUACCU U</p> <p>- UCU CGAA</p>

Fig. 7 (cont.)

miR-137	UAUUGCUUAAGAAUACGCGUAG	<p> G G A - GA CUUCGGU ACG GUUUCUUGGGUGG UAAUA CG \ GGAGCUU UGC CAUAGAUAUUGGU AUUGU GC U A G U AU </p>
miR-138	AGCUGGUGUUGUGAAUC	<p> -- UCA AC- C CG CAGCU GGUGUUGUGAA GGCCG GAG AG C GUUGG CCACAGCACUU UCGGC UUC UC A GA UA- CCA - CU </p>
miR-139	UCUACAGUGCAGUGUCU	<p> G - U A GUGGC GU UAUUCUA CAG GC CGUGUUCUCCAGU \ CA AUGAGGU GUC CG GCGCAGAGGUCG U - U C - GAGGC human </p>
miR-140	AGUGGUUUUACCCUAUGGUAG	<p> - A A UU UC CCUG CC GUGGUUUUACCCU HGUUAGG ACG A GGAC GG CACCAAGAUGGA ACCAUCU UGU U A - C -- CG </p>
miR-141	AACACUGUCUGUAAAGAUGG	<p> U -- U AU GARG GGG CCAUCUU CCAG GCAGUGUUGG GGUU \ CCC GGVAGRA GGUC UGUACACAAUC UCGA U - AU - C- AGUA </p>
miR-142s	CAUAAAGUAGAAGGCACUAC	<p> AC- A UAA--- G CCAUAAGUAG AAGCAGUAC CA C GGUAUUUCAUC UUUGUGAUG GU A GUA C UGGGAG C </p>
miR-142as*	UGUAGUGUUUCCUACUUAUGG	<p> AC- A UAA--- G CCAUAAGUAG AAGCAGUAC CA C GGUAUUUCAUC UUUGUGAUG GU A GUA C UGGGAG C </p>

Fig. 7 (cont.)

new	AUAAGACGAGCAGCAAAAGCUGU	<p> G G C C G G C AU UGAC GCGAGCUGUU GC CG UUAUAC UG \ ACUG UUGUUCGAAA CG GC AAUAUG AC G G A A AG C UC AL049829.4 </p>
miR-143	UGAGAUGAAGCACUGUAGCUa UUAGAUGAAGCACUGUAG	<p> G G U - AG CCUGAG UGCAGUGCU CAUCUC GG UC U GGACUC AUGUCACGA GUAGAG CU AG U G A U G GG AC008681.7 </p>
miR-144	UACAGUAUAGAUGAUGUACUAG	<p> G A A- GU GGCUGG AUAUCAUC UUAUCUGUA GUUU G CUGAUC UGUAGUAG AUAGACAU CAGA A A - CA GU </p>
miR-145	GUCCAGUUUCCAGGAUCCCUU	<p> C UC U C UGGAUG CUCA GG CAGU UU CCAGGAUCCCU \ GAGU UC GUCA AA GGUCCUAGGG C - UU U A UAGAAU </p>
miR-146	UGAGAACUGAAUCCAUUGGUUU	<p> CU C AUAUC AGCU GAGAACUGAAU CAUGGGUU A UCGA UUCUUGACUAA GUGUCCAG A C- A ACUGU </p>
miR-147	GUGUGUGGAAUUGCUUCUGCC	<p> A- CAA ACA--- GA AAUCUA AGA CAUUUCUGCACAC CCA \ UUAGAU UCU GUAAAGGUGUGUG GGU C CG UC- ACCGA AU human </p>
miR-148	UCAGUGCACUACAGAACUUUGU	<p> - A- CC - AGU GAGGCAAGUUCUG AG CACU GACU CUG \ CUCUGUUCAAGAC UC GUGA CUGA GAU A A AC -- A AGU human </p>

Fig. 7 (cont.)

miR-149	UCUGGCCUCCGUGUCUUCACUCC	<p> G C G A GUG G GGCUCUG CUC GU UCUC CUCCC UUU U UCGGGC GAG CA GGAG GAGG GAG C G A G - AG- C </p>
miR-150	UCUCCCAACCCUUGUACCAGUGU	<p> AC U UG- UG CCCUUCUCCCA CCU GUACCAG CUG \ GGAUAGGGGU GGA CAUGGUC GAC C CC - CCA UC </p>
miR-151	CUAGACUGAGGCCUUGAGGU	<p> C CA UGUCU CCUG CCUCGAGGAGCU CAGUCUAGUA \ GGAC GGAGUCCUGG GUCAGAUCAU C A A- CCCUC </p>
miR-152	UCAGUGCAUGACAGAACUUGG	<p> G A CC CCG C CCGGCCUAGGUUCUGU AU CACU GACU GCU U GGCCGGGUUCAGACA UA GUGA CUGA CGA G G C - - - - G </p>
miR-153	UUGCAUAGUCACAAAAGUGA	<p> GU A- AAU CAGUG UCAUUUUGUGAU UGCAGCU GU \ GUUAC AGUAAAACACUG ACGUUGA CG A U AU CC AGU </p>
miR-154	UAGGUUAUCCGUGUGCCUUCG	<p> U - CCU-- UUU GAAGUAGGUUA CCGUGU UG UCGC \ UUUUUAUCCAGU GGCACA AC AGUG A U U UAAGC UUU </p>
miR-155 [BIC-RNA]	UUAAUGCUAAUUGUGAUAGGGG	<p> U U A UUGGCC CUGUUAUUGCUAAU G G UAGGGUU \ GACAAUACGAUUG U C AUCCUCAG U - C - UCAGUC </p>

Fig. 7 (cont.)

name	sequence	structure
miR-C1	AACAUUCACGCGUCGGUGAGU	<pre> U A U CU A GGAUUC CCA GG ACA UCAACG GUCGGUG GUUU \ GGU CC UGU AGUUGC CAGCCAC CAAA A U A C -- - AAAACAAA </pre>
miR-C2	UUUGGCAUUGGUAGAACTUCACA	<pre> UU UGG UCA UAAGGU ACCAU UUGGCAA UAGAAC CACCGG A UGGUA AACCGUU AUCUUG GUGGCC A UC CAG --- CAGGGU </pre>
miR-C3	UAUGGCACUGGUAGAAUUCACUG	<pre> G AC-- GA -- AC CUGU UAUGGC UGGUA AUUCACUG UGA A GACA AUACCG GCCAU UAAGUGAC ACU G A GGAA -- UG CU </pre>
miR-C4	CUUUUUGCGGUCUGGGCUUGUU	<pre> - C CU G UUUU C UGGAU CUUUUUG GGU GGGUU CUG CU G AUCUA GAAAAAC CCA CCGAA GAC GA A U C UU G UGAU C </pre>
miR-C5	UGGACGGAGAACUGAUAAGGGU	<pre> U C AG -- UG CCU UCCUUAUCA UUUUCC CCAGC UUUU A GGA GGGAAUAGU AAGAGG GGUUG GAU C U C CA U CU </pre>
miR-C6	UGGAGAGAAAGGCAGUUC	<pre> A G AU UC AGGAUUGGAG GAAAG CAGUUCUG GG C UUCCUGGUCUC CUUUC GUCGGGAC CC C - G -- UC </pre>

Fig 7 (cont.)

name	sequence	structure
miR-C7	CAAAGAAUUCUCCUUUUGGGCUU	<pre> U UU UCUCAU ACUUUCCAAAGAAUUC CCUU GGGCUU U UGAAGGGUUUUUUAAG GGAA CCCGAA U U U- UUUUUAU </pre>
miR-C8	UCGUGUCUUGUGUUGCAGCCGG	<pre> A A C CGCUGC UC GGCU CAACACAGGAC CGGG U GG CCGA GUUGUGUUCUG GCUC C - C U CCCAGU </pre>
miR-C9	UAACACUGUCUGGUAAACGAUGU	<pre> C UU UUG GGGCAUC UUACCGGACAGUG UGGA UC CUUGUAG AAUGGUCUGUCAC AUCU AG G C A C- UUC </pre>
miR-C10	CAUCCCUUGCAUGGUGGAGGGU	<pre> CA UC GU UGAGCUC UCU CA CCUUGCAUG GGAGGG U AGG GU GGGACGUAC CCUCCC C AC UU AC CAAAAGU </pre>
miR-C11	GUGCCUACUGAGCUGACAUCAGU	<pre> G G A UA UCUCAU CUCC GU CCU CUGAGCUGA UCAGU U GAGG CA GGA GACUUGACU GGUCA C A A C C- CACACU </pre>
miR-C12	UGAUAUGUUUGAUAUAUUAAGGU	<pre> U- UA--- UU CUGUG GAUAUGUUUGAUAUAU GGUUG U GACAU UUAUACGAACUAUAUA CUAAU A CC UCAAC UU </pre>

Fig. 7 (cont.)

name	sequence	structure
miR-C13	CAACGGAAUCCCAAAAGCAGCU	<pre> C AGCGGG AACGGAUCC AA <u>AA</u> UU - C UCGUCC UUGCUUUAGG UU CGUCGAC UA GA A C - CA CU C G </pre>
miR-C14	CUGACCUAUGAAUUGACA	<pre> C UGACCUAUG AAUUG <u>CAGCCAG</u> G ACUGGAUAC UUAAC GUCGGUC U - C C UCCCCUC </pre>
miR-C15	UACCACAGGGUAGAACCACGGA	<pre> G UCCUG CCG UGGUUUUACCCU UGGUAGG ACG A AGGAC GGC ACCAAGAUGGGA <u>ACCAUCU</u> UGU U A - - C </pre>
miR-C16	AACUGGCCUACAAAGUCCCG	<pre> A U C A A AGU GAG GCUGGG CUUUG GGGC AG UGAG G CUC UGACCC <u>GAAAC UCCG UC ACUU</u> U C U A G A GAC </pre>
miR-C17	UGUAAACAGCAACUCCAUUGUGGA	<pre> U A G - - U AUCGGG GUAACAGCA CUCCAU <u>UGGA</u> CUG G UAGUCU CAUUGUCGU GAGGUG ACCU GGC C U C - UA U </pre>
miR-C18	UAGCAGCACAGAAAUUUGGC	<pre> U A- AGCAGCACAG AAUAUUGGCA GG G UCGUCGUGUC UUAUAACCGU CU U GG - - GAG </pre>

Fig 7 (cont.)

name	sequence	structure
miR-C19	UAGGVAGUUUCAUGUUGUUGG	<pre> A A C GCCCUGGG GUGAAUU GGU GUUU AUGUUGUUG U CACUUG CCA CAAA UACAACAAC U C C C U ACAAGUCU </pre>
miR-C20	UUCACCACCUUCUCCACCAGC	<pre> C A CA GA - A GGCUGGC GGU GAGAGG GUGG GGU AAG G CCGGUAG CCCA CUCUCC CACU CCA UUC C A C AC UC C U </pre>
miR-C21	GGUCCAGAGGGGAGAUAGG	<pre> G - C G U UCCUG UCAUU G UC A AGGGAGA AGG U AGUAA U AG U UCUCUUCU UCC G A A A A - UUUUUA </pre>
miR-C22	CCCAGUGUUCAGACUACCUGUU	<pre> AAC U C U G--- G GCC CCAGUU CAGACUAC UGU CA GAG \ CGG GGUACA GUCUGAUG ACA GU CUC C AUU C - U GUAA U </pre>
miR-C23	UAAUACUGCCUGGUAUGAUGAC	<pre> GGC - C UAGUG GCCGU CAUC UUAUUGGCAG AUUGGA U CGGCA GUAG AAUGGUCCGUC UAAUCU C --- U A CUAGU </pre>
miR-C24	UACUCAGUAGGCAUUGUUCU	<pre> U U UUC A UACCUUAC CAG AAGGCAUUGUUC UAU U AUGGGAUG GUC UUCGUGACAAG AUA U U U UAA A </pre>

Fig. 7 (cont.)

name	sequence	structure
miR-C25	AGAGGUUAUAGCGCAUGGGAAGA	<pre> U A- UG C GUUCC UUUUCCUAUGC UAUACUUCUU UGGAU \ CGAGG AGAAGGGUACG ADAUGGAGAA AUUCU U U CG -- G </pre>
miR-C26	UGAAAUUUUAGGACCACUAG	<pre> C U G A C U GGUC AGUGGUUCU GACA UUCA CAGUU UG \ CCAG UCACCAGGA UUGU AAGU GUUAA AC A A U A - C G </pre>
miR-C27	UUCCCUUUUGUCAUCCUAUGCCUG	<pre> U A U GAGAAUA UGGAC UCCCUUUGUC UCCUA GCCU \ ACUUG AGGGAACCG AGGGU CGGA U C A - GGAAGUA </pre>
miR-C28	UCCUUCAUUCCACCGGAGUCUG	<pre> UC C UCUUA CUCUUG CUUCAUCCAC GGAGUCUG U GAGGAC GAAGUGAGGUG CUUUGAGC G UC A CAAC </pre>
miR-C29	GUGAAAUUUUAGGACCACUAGA	<pre> U C U G A C U GCC GGUC AGUGGUUCU GACA UUCA CAGUU UG \ CGG CCAG UCACCAGGA UUGU AAGU GUUAA AC A C A U - C G </pre>
miR-C30	UGGAAUGUAAGGAAGUGUGUGG	<pre> - C U AUUUC CCAGG CCACAUGCUUCUUUAU C CAUAG \ GGUU GGUGUGGAAGGAAGUA G GUUUC U U A - ACGAC </pre>

Fig 7 (cont.)

name	sequence	structure
miR-C31	UACAGUAGUCUGCACAUUGGUU	<pre> AUC U C ----- G GCC CCAGUGU CAGACUAC UGU UCAG A CGG GGUUACA GUCUGAUG ACA GGUC G AUU C - UGUACAG G </pre>
miR-C32	CCCUAGAGAACCGAAUUUGUGU a miR-10 variant	<pre> A G C DG- AC UAUUA CCCU UAGAA CGAAUUUGUG GU C AUAUA GGGG AUUUU GCUUAGACAC UA C A - A UGA CA </pre>
miR-C33	AACCCGUAGAUCCGAACUUUGUGA A a miR-99a variant	<pre> A C C A C AU CACA ACC GUAGAU CGA CUUGUG UG U GUGU UGG UAUCUG GUU GAACAC AC C A A U C - GU </pre>
miR-C34	GCUUCUCCUGGCUCUCCUCCUCCUC	<pre> C U UUG GGAG AAGG AGGGG GAGGGG CCGGAGGAGC CGGGC G UUCC UCUCU CCUCUC GUCCUCUUCG GUUCG C - - UCG C GCGU </pre>

Fig. 7 (cont)

name	human	C.elegans	mouse								Drosophila	fugu fish	zebrafish
			liver	small intes	colon	cerebellum	cortex	midbrain	heart	spleen			
let-7a-1	AC007924 chr9 AC087784 chr 17 identical precursor		num.hits in trace data, 3 families of similar precursors		found		nearly identical precursor	found					
let-7a-2	AF001339 chr11						nearly identical precursor						
let-7a-3	AL049853 chr22	AP374345 chrX with diff. precursor									AB003659 diff. precursor		
let-7b	AL049853 chr22		nearly identical precursor			nearly ident precursor trace#4811003		found		EST AK481799.1 spleen cerebellum (mammary)		with slightly diff precursor	
let-7c	AB001667 chr21		identical and diff. precursors			num.genomic hits, ident precursor/diff precursor -> EST A1614897	numerous genomic hits	found					
let-7d	AC007924.3 chr9 AC087784 chr17 identical				found	trace#83587042 nearly ident prec	trace#83587042 nearly ident prec	found	found				
let-7e	AC018755 chr19							found		FOUND			
let-7f-1	AC007924 chr9 AC087704 chr17					ident precursor genomic DNA		found	found				
let-7f-2	AL392016 chrX					ident. precursor in mntase 18713911							
let-7g	precursor ident. to mouse in AC092045.2 chr3					genomic hits, no EST		found					
let-7h							found in cortex, no db hit						

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

	19, AC020916				found	found, but no db hit	found, but no db hit for mouse	found	found, maps to chr 13 NGSC mntace 44671617	found	found			
mir-27a														
mir-27b	X4_090911.1 chr9 identical precursor													
mir-28	3, AC063932													
mir-29a	7, AF017104 second ident. copy found in chr7 cluster, this cluster also coaxed in mouse: AC014913.32				found, AC024913.3 2	found, mntace123467334	nearly ident precursor trace12346733 4, EST AC014913.32			trace, EST, nearly ident prec				
mir-29b	AC014913.32 CLUSTER of mir-29-b and 29-c) miRNA similar to mir-83				found		AC024913.321d found 1st precursor in EST B014396 (testina) found			FOUND			scaffold-17670 (A third copy)	
mir-29c										found, supported by ESTs			scaffold-17670 has two copies of this RNA	
mir-30a-s	nearly ident fold in AL033467.23 chr6						found		found					
mir-30a-ss														
mir-30b	human AP15927.6 chr8 different precursor					found with diff. precursor in trace 185261735								
mir-30c	AL136164.8 chr.6 supported by ESTs (BF594736.1)					trace172329251	found						scaffold-3483, diff precursor	

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

miR-124a	nearly ident. precursor in chr8(AC01518) chr20(AL096828)	found in 372504.1 chr1V intron, diff precursor	found		most abundant in most cereb., genomic hits (trace121097008, 11737241) found, but no db hit	found A12U	found in AC006590.1 1 with diff fold	slightly diff precursor AC009251 chr2L		
miR-124b	AC01518 chr8, nearly ident chr20 AL096828.29									
miR-125a	ident. precurs in AC016755.3 chr 19				found					
miR-125b	AP001359.4 chr11 AP001657.1 chr21(chr21 like mouse)				trace18398570 5	found with A12U	found in AC006590.1 1 with diff fold			
miR-126					untrace1351397 and more	found	with diff precursor affold_32 95			
miR-127	human AL117190.6 chr.14 same precurs as in mouse				hit in trace179514837					
miR-128	ident in AC016742.10 chr 2, diff prec in AC016943.7 chr.3				genomic hit trace151670230	found	Scaffold 828, diff prec			
miR-129	human AC018662.3 chr7				found, but no db hit					
miR-130					untrace 6847278				with diff fold AC091399.2	
miR-131	AC00317.2 chr 15 align, diff precursor, but AC026701.6 chr 5 ident				several trace hits, mouse AP155142	found				
miR-132	AL137038.5 chr17 prec align, diff from mouse				trace hit186984641					

Fig. 7 (cont.)

mir-133	AC039221.15 chr6 diff. Precursor(ident. to rat L33722.1)					found, trace 62407955	found	AC039440.1 diff. Precursor	Scaffold_1049; pre- u nearly like mouse	
mir-134	AC137708.5 chr14 similar precursor					1 trace1642031				
mir-135	AC032013.2 chr3 AC018659.35 chr12 (ident or small to mouse)					5, EST trace1719323 5, EST 1(kidn., sple en) (=chr3 huma n)	found		Scaffold_2125 with similar precurs	
mir-136	AL117190.5 chr14 ident to mouse					3 trace18607175				
mir-137	AC037691.1 chr1 ident to mouse, nearly ident fish					3, EST trace18977454 (hypothal) A18 52436.1, ident			Scaffold_18244 nearly ident to mouse/man	
mir-138	AC006058.1 chr3 precursor diff					mouse EST 3852820.2				
mir-139	AP001065.2 chr11					found, but no mouse hit				
mir-140	AC026468.8 chr.16, precursor r nearly ident,				several trace hits; trace11053 0193					
mir-141	AC006512.12 chr12, precursor slight diff				AC002197 chr6		found			
mir-142s	AC004687.1 chr17 BCL3/myq translocation locus, like mouse				found					
mir-142ss*					several EST A183235		found			

Fig. 7 (cont.)

[illegible]

Fig. 7 (cont.)

mir-153	AC006372.1 chr7 ident. precursor									found sever. mmtrace 87010874						
mir-154	AL32709.5 chr14 nearly identical precursor									found sever. mmtrace 96718639						
mir-155 (BIC-RNA)	human_BIC RNA:AF402776.1 (has U2C)								found chr 16 mouse							

Fig. 7 (cont.)

name	human	mouse						Drosophila	fugu fish	zebrafish
		spleen	eye	kidney	testes	lung	thymus	skin		
mir-C1	with different precursors in chr9 AL158075.11, chr1 AL136321.5		mouse trace #76647842			found		found	scaffold_1819	
mir-C2	chr7 AC084864.2 similar precursor		mouse trace #8841093						scaffold_967	AL590150.2
mir-C3	chr7 AC084864.2 ident. precursor		trace #86029980						scaffold_967	AL590150.2
mir-C4	similar precursor in chr7 AC018662.3		trace #13885686		found					
mir-C5	chr15 AC069082.9		trace #87318220						scaffold_3671	
mir-C6	chr22 AC005664.2 ident. precursor		chr15 AC013526.32							
mir-C7	chr1 AL512443.7 similar prec.		trace #86694995							
mir-C8				found, trace #51673394						
mir-C9				found, trace #78364603					scaffold_2310, diff. precursor	
mir-C10	chrX AF222686.1 nearly ident. precursor			found, trace #61928192						
mir-C11	chr9 XM_028943.1 has chr17; prec. nearly identical to mouse			found, cDNA AL286629.1. has chr17						
mir-C12				found, trace #71760650					scaffold_2394	
mir-C13		found		found, trace #8872637						

Fig. 7 (cont.)

name	human	mouse						Drosophila	fugu fish	zebrafish
		spleen	eye	kidney found, but no db hit	testes	lung	thymus			
miR-C14	chr11 AC000159.6									
miR-C15	chr16 AC026468.6 nearly ident. precursor			EST BX687377.1, several trace					scaffold_2083	
miR-C16	chr17 AC00101.1, similar precursor			found, trace#95 55103					scaffold_246	
miR-C17	chr11 AC000159.6, chr1 AC103590.2, diff. prec.			found, trace #97796402					scaffold_152	
miR-C18				found, trace #47821788 (close to mir- 14)		found				
miR-C19	chr17 AC009749.21 cloned from human cell line only			similar precursor in mouse chr1 AC011494.15					scaffold_28334	
miR-C20	chr1 AL355110.19 cloned from human cell line only									
miR-C21	chr3 AC063952.15 cloned from human cell line only									
miR-C22	chr19 AC007229.1, chr1 AL137157.7 similar precursor/ cloned from human cell line only								scaffold_8399	
miR-C23					trace #72257777	found			scaffold_2210	
miR-C24					trace #69679879					
miR-C25					trace #49754566					
miR-C26	AL136001 ident. precursor				trace #11977216					

Fig. 7 (cont.)

name	human	mouse					Drosophila		fugu fish	zebrafish
		spleen	eye	kidney	testes	lung	thymus	skin		
mir-C27	chr9 AL159990.12 identical precursor		trace #91503159						scaffold_725	
mir-C28	XN_036612.4, precursor very similar							XN_149012.1	scaffold_13664	
mir-C29	chr14 AL136001.6 nearly identical precursor							trace #18453604		
mir-C30	chr6 AL391231.15 similar precursor							trace #84055510		
mir-C31	chr9 AC006312.8							trace #89079710	scaffold_5830	
mir-C32								U7364.1, intronic location Hoxd4 gene	scaffold_82	
mir-C33								trace #84780844	scaffold_15612	
mir-C34							trace 73109322			

SEQUENCE LISTING

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<151> 2001-09-28

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22

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Oligonucleotide

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23

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Oligonucleotide

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Oligonucleotide

<400> 15
gcaagaactc agactgtgat g 21

<210> 16
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Oligonucleotide

<400> 16
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<210> 17
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22

<210> 18

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<400> 18

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<210> 28

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<210> 33

<211> 22

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<210> 34

<211> 22

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<210> 35

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21

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<213> Artificial Sequence

<220>

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Oligonucleotide

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gcttccagtc gaggatgttt aca

23

<210> 41

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

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<400> 41

cgcaagggtcg gttctacggg tg

22

<210> 42

<211> 20

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<220>

<223> Description of Artificial Sequence:

Oligonucleotide

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20

<210> 43

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<213> Artificial Sequence

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acaaacacca ttgtcacact cca

23

<210> 44

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<212> DNA

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<400> 44

tggcattcac cgcgtgcctt a

21

<210> 45

<211> 23

<212> DNA

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<223> Description of Artificial Sequence:
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<400> 45

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23

<210> 46

<211> 22

<212> DNA

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<223> Description of Artificial Sequence:
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<210> 48
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<210> 49
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<223> Description of Artificial Sequence:
Oligonucleotide

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gcaagcccag accgaaaaa g

21

<210> 50
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<223> Description of Artificial Sequence:
Oligonucleotide

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<210> 51

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<210> 52

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23

<210> 53

<211> 22

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<223> Description of Combined DNA/RNA Molecule:
Oligonucleotide

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22

<210> 54

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18

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<210> 63

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<400> 63

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uagcagcaca uaaugguug ug

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